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Insertion sequence IS*200* fingerprinting of *Salmonella typhi*: an assessment of epidemiological applicability

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SUMMARY

When *Pst* I-generated digests of genomic DNA from each of the type strains of 49 of the Vi phage types of *Salmonella typhi* were probed with a PCR-amplified IS*200* gene probe, all strains were found to possess at least 11 IS*200* elements carried on fragments in the range 24·2–1·2 kb. Fourteen fingerprints were identified but two patterns designated IS*200Sty*1 and IS*200Sty*2 predominated. In one strain, a plasmid-mediated IS*200* element was identified. When IS*200* fingerprinting was applied to epidemiologically-unrelated strains of *S. typhi* isolated in Ecuador, 3 patterns were identified in 10 strains belonging to 9 different phage types. It is concluded that Vi phage typing remains the method of choice for the primary differentiation of *S. typhi* but that IS*200* fingerprinting may be of limited use in laboratories which do not have access to phage typing.

INTRODUCTION

The mobile genetic element IS*200*, first described in 1983 by Lam and Roth [1] has been shown to be distributed on conserved loci on the chromosome of many salmonella serotypes [2] but has not been specifically identified in other members of the Enterobacteriaceae [3]. Because IS*200* elements were identified in the genotype of strains belonging to 22 of 23 salmonella serotypes (other than *Salmonella arizonae*) [2], it was suggested that IS*200* might be a suitable probe to identify clinical isolates of salmonella with relatively high accuracy [2].

Unlike other enterobacterial insertion elements such as IS*1* and IS*5*, IS*200* transposes only rarely under laboratory conditions [4] and this stability has favoured its use as a probe for discrimination within serotype, and for phylogenetic purposes. The distribution of IS*200* elements in the genotype has therefore been used to define clonal lineages within serotypes such as *S. enteritidis* [5], *S. berta* [6] and *S. bovis-morbificans* [7] and in *S. heidelberg*, the copy number of IS*200*

elements was high enough to provide a molecular fingerprint suitable for epidemiological purposes [8]. Within phage typable serotypes, IS*200* typing was not sufficiently discriminatory for the primary subdivision of *S. enteritidis* [5, 9] and *S. virchow* [10]. In contrast, eight IS*200* profile patterns have been identified within strains belonging to 9 of the 10 common phage types of *S. typhimurium* [11], and it has been suggested that IS*200* typing may be suitable for the genotypic subdivision of this serotype [11].

In their study of the distribution of IS*200* elements in a range of different salmonella serotypes, Gibert and colleagues demonstrated that the number of IS*200* elements in 15 strains of *S. typhi* varied from 10–25 [2]. The high copy number of elements coupled with considerable variation in the numbers identified in different strains suggests that IS*200* fingerprinting may be used for the genotypic subdivision of this clinically important serotype. The internationally accepted method for the primary subdivision of *S. typhi* is Vi-phage typing and using the method of Craigie and Felix [12], over 100 phage types have now been recognized [13]. However, it has recently been demonstrated that when applied to representative drug-resistant and drug-sensitive strains of *S. typhi* belonging to Vi-phage types A, E1 and M1, IS*200* fingerprinting provided a method of discrimination within phage types E1 and M1 [14] and may therefore provide an extra dimension to Vi-phage typing.

We now report the distribution of IS*200* elements in the individual type strains of 49 of the Vi phage types of *S. typhi*. The application of IS*200* fingerprinting to the discrimination of epidemiologically-unrelated strains of *S. typhi* isolated in Ecuador between 1990 and 1992 is also described and compared to phage typing, and the potential of this method of genotypic fingerprinting for the primary subdivision of *S. typhi* is discussed.

MATERIALS AND METHODS

Bacterial strains and culture methods

The type strains of the *S. typhi* Vi-phage types studied were those of phage types A, B1, C1, D1, E1, F1, G1, H, J1, K1, L1, M1, N, O, T, 25, 26, 27, 28, 29, 32, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60 and 61. These strains had been maintained at 4 °C on Dorsets' egg agar in the culture collection of the Laboratory of Enteric Pathogens (LEP). The strains of *S. typhi* from Ecuador had been isolated from patients in the 3-year period 1990–2 and as far as is known, were from epidemiologically-unrelated cases. Strains were phage-typed by the methods of Craigie and Felix [12] and for the extraction of genomic DNA, were incubated at 37 °C in 3 ml Nutrient Broth for 18 h, with gentle aeration. All manipulations with live cultures of *S. typhi* were carried out in the P3 Containment Facility of LEP.

Genomic DNA preparation

Genomic DNA prepared in accordance with the method of Ausubel and colleagues [15] was precipitated by the addition of 0·6 volumes of isopropanol and the solution allowed to stand for 15 min before centrifugation. The resultant DNA was then washed with 70% ethanol, dried and treated with RNAse (final

concentration 50 ng/μl in TE: 10 mM Tris-HCl; 1 mM EDTA; pH 8·0) for 30 min at 37 °C. DNA concentrations were quantified spectrophotometrically and 10 μg quantities of the resultant preparations were digested to completion with *Pst* I (Gibco BRL), electrophoresed and transferred to Hybond Nylon Hybridization membranes (Amersham UK) by vacuoblotting as described by Torre and colleagues [14].

*Preparation and use of IS*200 *probe*

The IS*200* probe, a multimer of the 100 bp *Taq* I–*Taq* I fragment of IS*200*, was amplified by PCR using a Cambio Mark II Thermocycler and the following amplification cycle: 94 °C, 1 min; 55 °C 1 min; 72 °C, 1 min; 35 times. The substrate was plasmid pUA175, which is a pUC19 derivative containing an internal 100 bp *Taq* I–*Taq* I multimer of IS*200* cloned into the *Eco*R I site [16]. After amplification, the PCR product was checked by agarose gel electrophoresis and labelled with digoxygenin according to the instructions of the manufacturer (Boehringer Mannheim). Hybridization procedures were as described previously [14] and the final concentration of probe used for hybridization was 25 ng per ml of hybridization fluid. Bands were sized in relation to digoxygenin-labelled λ DNA digested with *Hin*d III, and *Pst* I-digested genomic DNA from *S. typhimurium* strain LT2.

Extraction of plasmid DNA

Partially-purified plasmid DNA was extracted by a modification of the method of Kado and Liu [17] and after electrophoresis on 0·7% Tris-Borate agarose gels, was transferred to Hybond membranes by vacuoblotting (LKB vacu-gene apparatus) for testing with the PCR-amplified, IS*200* gene probe.

RESULTS

*IS*200 *elements in* S. typhi *Vi-phage type strains*

Between 11 and 15 IS*200* elements were identified in *Pst* I-digested genomic DNA from the 49 type strains of the Vi-phage types listed above. IS*200*-carrying bands ranged in size from 24·2–1·21 kb and 13 patterns were identified (Fig. 1, gels A, B, C). In accordance with the scheme of Threlfall and colleagues for the designation of *S. typhi* IS*200* fingerprint patterns [14], these patterns were designated IS*200Sty*1 through to IS*200Sty*14. The pattern designated IS*200Sty*3 previously identified in drug-resistant strains of Vi-phage type M1 [14] was not observed in this study.

The most common pattern was that designated IS*200Sty*1 (for example, see Fig. 1, gel A, lane 1). This profile is comprised of 2 upper bands of approximately 22·8 and 21·4 kb, a third band of 16·4 kb, 3 middle bands of 10·2, 9·8 and 9·2 kb, 5 lower bands of 5·4, 5·0, 4·3, 3·9 and 3·6 kb, and a final band of 2·3 kb. With 5 exceptions, the 2 upper bands, the third band of 16·4 kb, the 3 middle bands, 4 of the 5 lower bands with MWs of 5·4, 5·0, 4·3 and 3·9 kb and the final band of 2·3 kb were conserved in *Pst* I digests of all the Vi type strains tested. The four exceptions were the pattern IS*200Sty*5, which lacked the lower band of 5·4 kb (for example, see Fig. 1, gel A, lane 15 – Vi phage type O), IS*200Sty*10, which lacked the final band of 2·3 kb (Fig. 1, gel C, lane 3 – Vi-phage type 47), IS*200Sty*12, which lacked

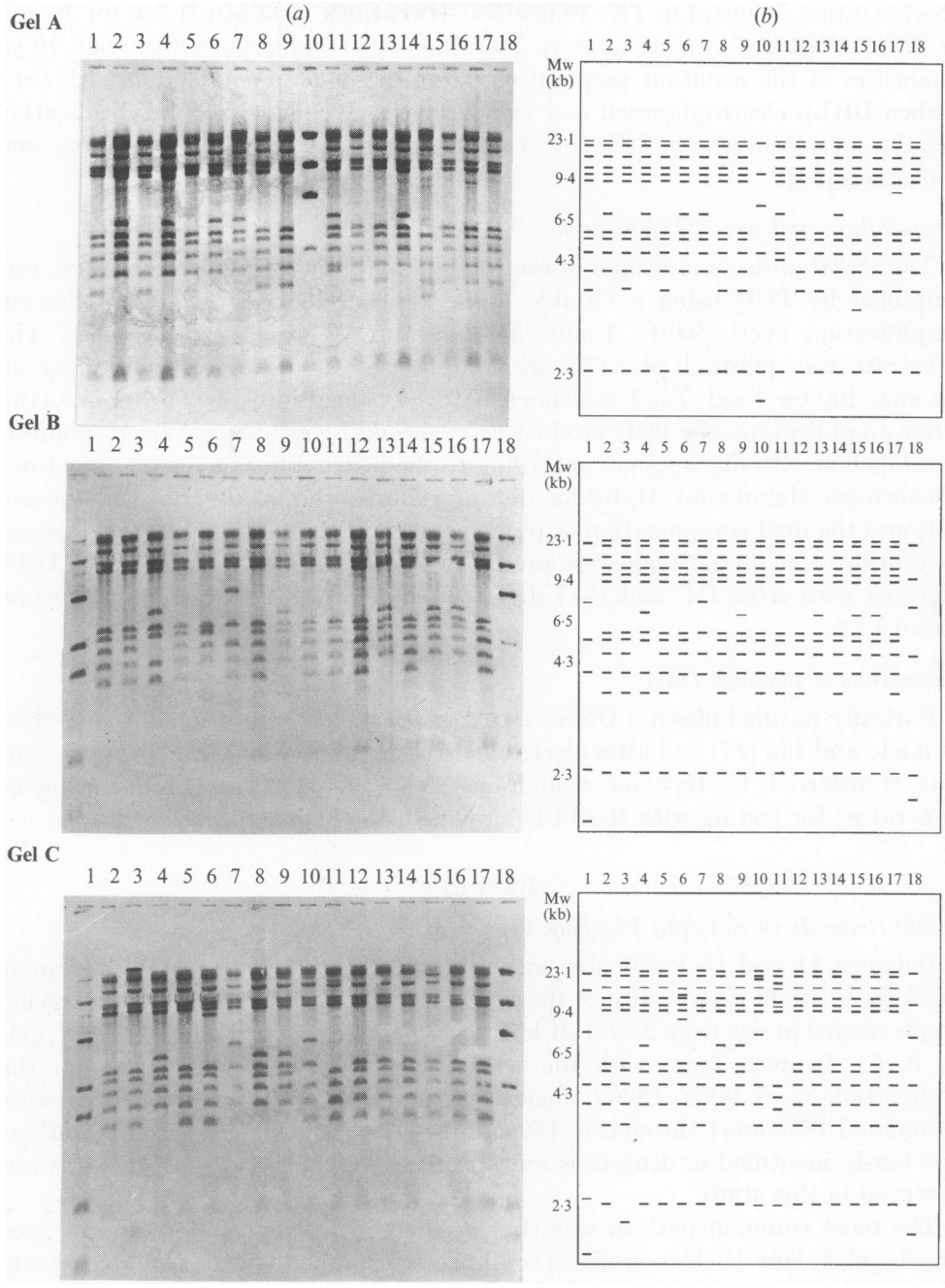

Fig. 1. Insertion sequence IS*200* fingerprints of *Pst* I-generated digests of genomic DNA of type strains of *Salmonella typhi* Vi-phage types (*a*) and (*b*) diagrammatic representation. **Gel A**: Lane 1, Vi-phage type A; 2, B1; 3, C1; 4, D1; 5, G1; 6, F1; 7, G1; 8, H; 9, J1; 10, digoxygenin-labelled λ DNA digested with *Hin*d III; 11, K1, 12, L1; 13, M1; 14, N; 15, O; 16, T; 17, 25; 18, 26. **Gel B**: Lane 1 genomic DNA from *S. typhimurium* LT2 digested with *Pst* I; 2, Vi-phage type 27/*Pst* I; 3, 28; 4, 29; 5, 32; 6, 34; 7, 35; 8, 36; 9, 37; 10, 38; 11, 39; 12, 40; 13, 41; 14, 42; 15, 43; 16, 44; 17, 45; 18, λ/*Hin*d III. **Gel C**: Lane 1, *S. typhimurium* LT2/*Pst* I; 2, Vi-phage type 46/*Pst* I; 3, 47; 4, 48; 5, 49; 6, 50; 7, 51; 8, 52; 9, 53; 10, 54; 11, 55; 12, 56; 13, 57; 14, 58; 15, 59; 16, 60; 17, 61; 18, λ/*Hin*d III.

Table 1. *Distribution of IS*200 *profile patterns in type strains of 49* S. typhi *Vi-phage types*

IS*200* profile pattern	Vi-phage type
IS*200Sty*1	A, C1, E1, H, J1, L1, M1, T, 26, 27, 28, 32, 36, 38, 39, 40, 42, 45, 46, 49, 56, 57, 58, 60, 61
IS*200Sty*2	B1, D1, F1, G1, N, 37, 41, 43, 44, 48, 52, 53
IS*200Sty*4	K1
IS*200Sty*5	O
IS*200Sty*6	25
IS*200Sty*7	29
IS*200Sty*8	34
IS*200Sty*9	35
IS*200Sty*10	47
IS*200Sty*11	50
IS*200Sty*12	51
IS*200Sty*13	54
IS*200Sty*14	55
IS*200Sty*15	59

the lower band of 5·0 kb (Fig. 1, gel C, lanes 7 and 10 – Vi-phage types 51 and 54), IS*200Sty*13, which lacked the lower band of 5·4 kb (Fig. 1, gel C, lane 11 – Vi-phage type 55) and finally IS*200Sty*14, which lacked the upper band of 22·8 kb (Fig. 1, gel C, lane 15 – Vi-phage type 59).

The second most common profile type was that designated IS*200Sty*2 (for example, see Fig. 1, gel A, lane 2). This profile can be differentiated from IS*200Sty*1 by the presence of an additional band of 6·0 kb and the absence of the band of 3·6 kb. The remaining eight IS*200* profiles were characterized by the presence or absence of bands ranging in size from approximately 20 kb (IS*200Sty*11 – Fig. 1, gel C, lane 6 – Vi-phage type 50) to 3·2 kb (IS*200Sty*4: Fig. 1, gel A, lane 11 – Vi-phage type K1). IS*200Sty*10 was exceptional in that, although lacking the final band of 2·3 kb, the pattern was characterized by an additional small band of 2·0 kb and an additional large band of approximately 25 kb (Fig. 1, gel C, lane 3). The latter band was thought to be related to the possession of an IS*200*-carrying plasmid (see below).

*Distribution of IS*200 *profiles in* S. typhi *Vi-phage types*

The distribution of IS*200* profiles in the 49 *S. typhi* Vi-phage type strains studied is shown in Table 1. The two profile types IS*200Sty*1 and IS*200Sty*2 predominated, comprising respectively 51 and 24% of the type strains tested. The IS*200Sty*1 group contained the type strains of the common Vi phage types A, E1, J1, M1 and 46 and the IS*200Sty*2 group, the type strains of Vi phage types B1, D1 and F1. Only one representative of each of the remaining 12 profile types was identified.

Plasmids in type strains S. typhi *Vi phage types*

Nineteen of the 49 Vi phage type strains studied carried plasmids with MWs ranging from approximately 150–4·5 kb. Of these strains, 7 carried a plasmid of approximately 36 kb, 6 a plasmid of 90 kb and 2, a plasmid of 4·5 kb. In the

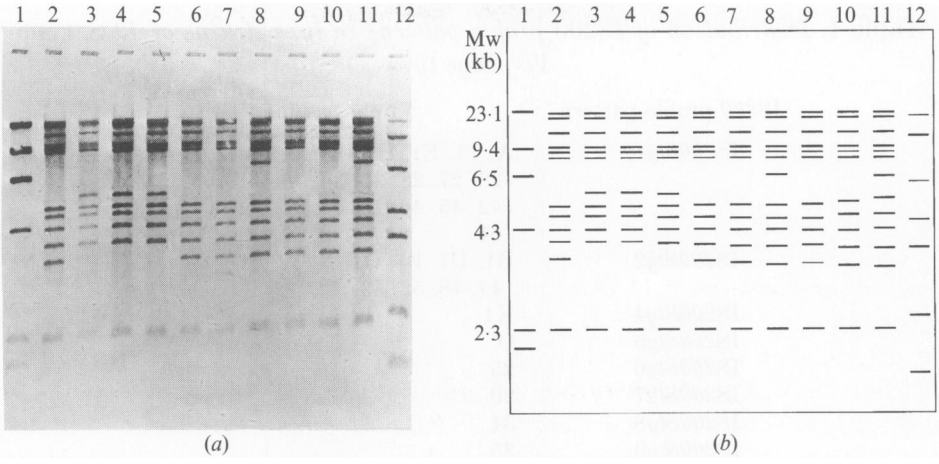

Fig. 2. IS*200* fingerprints of strains of *S. typhi* isolated in Ecuador (*a*) and (*b*) diagrammatic representation. Lane 1, Digoxygenin-labelled λ DNA digested with *Hind* III; 2, P227485/*Pst* I; 3, P227582; 4, P227507; 5, P227579; 6, P277486; 7, P277586; 8, P277522; 9; P227615; 10, P227487; 11, P277597; 12, *S. typhimurium* LT2/*Pst* I.

Table 2. *Phage types and IS*200 *profile patterns in* S. typhi *from Ecuador*

Lab reference	Vi-phage type	IS*200* profile pattern
P227485	A	IS*200Sty*1
P227582	E7	IS*200Sty*2
P227507	F1	IS*200Sty*2
P277579	G1	IS*200Sty*2
P277486	M1	IS*200Sty*1
P277586	46	IS*200Sty*1
P277522	DVS	IS*200Sty*6
P277615	DVS-5	IS*200Sty*1
P227487	DVS-14	IS*200Sty*1
P277597	DVS-14	IS*200Sty*6

remaining 4 plasmid-carrying strains, plasmids ranging from 150–20 kb were identified.

In only one plasmid-carrying strain, the type strain of Vi phage type 47, was hybridization with the IS*200* probe observed. In this strain, strong hybridization with a plasmid of approximately 90 kb was detected (data not shown). The resultant hybridization band of approximately 30 kb can be seen in *Pst* I digests of total genomic DNA (Fig. 1, gel C, lane 3).

*Phage types and IS*200 elements in S. typhi *from Ecuador*

When IS*200* fingerprinting was applied to ten epidemiologically-unrelated strains of *S. typhi* belonging to nine different phage types which had been isolated in Ecuador between 1990 and 1992, three patterns of hybridization were observed, IS*200Sty*1, IS*200Sty*2 and IS*200Sty*6, with the IS*200Sty*1 and IS*200Sty*2 patterns predominating (Fig. 2, Table 2). The IS*200Sty*1 pattern was found in five strains which belonged to five different phage types comprising the Vi-phage types A, M1 and 46 and the degraded types DVS-5 and DVS-14. The IS*200Sty*2 pattern was

identified in three strains of phage types E7, F1 and G1 and the IS*200Sty*6 pattern in the two remaining degraded Vi types, DVS and DVS-14. All ten strains of *S. typhi* from Ecuador were plasmid-free.

DISCUSSION

In this study, hybridization analysis using a PCR-generated IS*200* gene probe has demonstrated that all of the type strains of the 49 Vi-phage types of *S. typhi* examined possess at least 11 IS*200* elements carried on *Pst* I-generated fragments ranging from 24·2 to 1·21 kb. The number of bands carrying IS*200* elements varied from 11–15. Fourteen distinct profile types have been identified, designated IS*200Sty*1 through to IS*200Sty*15 in accordance with the scheme of Threlfall and colleagues [14]. The pattern designated IS*200Sty*3, previously identified in drug-resistant strains of *S. typhi* belonging to Vi-phage type M1 was not observed in this study. Within the profile patterns identified, some IS*200*-carrying bands appeared to be highly conserved whilst others were only partly conserved. For example, bands of 21·4, 16·4, 10·2, 9·8 and 4·3 kb were conserved in all digests whereas bands of 22·4, 5·4, 5·0, 3·9 and 2·3 kb were only partly conserved.

Previous studies have demonstrated that the fingerprint designated IS*200Sty*1 probably represents the archetypal *Pst* I-generated IS*200* fingerprint for *S. typhi* [14]. The current study has demonstrated that this fingerprint, originally identified in the type strains of Vi-phage types A, E1 and M1, is also provided by a further 20 of the 49 Vi phage strains. IS*200Sty*1 is characterized by 12 IS*200* elements carried on fragments ranging from 22·8–2·3 kb. The next most common pattern, IS*200Sty*2, first identified in a drug-sensitive strain of Vi-phage type E1 isolated in India [9] was provided by a further 12 of the Vi phage type strains. This pattern is closely related to IS*200Sty*1 and appears to have been derived from IS*200Sty*1 by intragenomic rearrangement, possibly resulting from loss of a *Pst* I site in the genome. Thus, IS*200Sty*2 can be differentiated from IS*200Sty*1 by the presence of an additional IS*200*-carrying band of 6 kb and the absence of a band of 3·6 kb. Eight of the remaining ten IS*200* patterns appear to have been derived from IS*200Sty*1 by the acquisition, either by genomic rearrangements or transposition, of one or more additional elements carried on bands ranging from 20–6·2 kb, coupled with the loss of IS*200*-carrying bands of 5·4, 5·0 and 3·6 kb. However two patterns were exceptional in that they appear to have been derived from IS*200Sty*1 either by the complete loss of the highly conserved upper band of 22·8 kb – for example IS*200Sty*15, or by the acquisition of a plasmid-mediated IS*200* element – for example IS*200Sty*10. To our knowledge this is the first notification of a plasmid-mediated IS*200* element and further studies to characterize both the plasmid and the IS*200* element encoded by this plasmid are in progress.

The pattern previously designated IS*200Sty*3 could be differentiated from IS*200Sty*1 by the possession of an additional IS*200* element carried on a band of 19·0 kb [14]. Although the IS*200Sty*3 pattern was not observed in the strains studied here, an additional band of approximately 19·0 kb was identified in strains with the IS*200Sty*12 pattern. However, unlike strains with the IS*200Sty*3 pattern, strains with the IS*200Sty*12 and IS*200Sty*13 patterns lacked the lower band of

5·0 kb but possessed an additional band of 8·0 kb, possibly resulting from spontaneous loss of a *Pst* I site in the genome of the type strains of Vi-phage types 51 and 54.

It has been suggested that for *S. typhimurium*, *IS200* fingerprinting may be as discriminatory as phage typing for the primary subdivision of the serotype [11]. In contrast to these findings, the results presented above demonstrate that for *S. typhi*, IS*200* fingerprinting cannot be used as substitute for Vi-phage typing for the primary discrimination of *S. typhi*, despite the high copy number of the element in the genome. In particular, although 14 patterns of hybridization were identified within the 49 Vi phage type strains tested, 25 strains (51%) were characterized by the IS*200Sty*1 pattern and 12 (24%) by the IS*200Sty*2 pattern. Strains with the IS*200Sty*1 pattern included the type strains of Vi-phage types A, E1, J1, M1 and T, all Vi-phage types common in patients infected in the Indian sub-continent and South-east Asia and also Vi-phage type 46, which is common in some South American countries. When applied to epidemiologically-unrelated strains of *S. typhi* from Ecuador, the limited potential of IS*200* fingerprinting for the primary differentiation of this serotype was particularly evident, when only three IS*200* profile types were identified in ten strains belonging to nine different phage types.

We therefore conclude that Vi-phage typing remains the method of choice for the primary differentiation of *S. typhi* but that IS*200* fingerprinting may be of limited use in laboratories which do not have access to phage typing. These studies also demonstrate that without epidemiological validation, molecular methods of genotypic typing such as IS*200* fingerprinting should be used in conjunction with more traditional methods of strain discrimination such as phage typing and not as an alternative method of typing.

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