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# Subdivision of *Salmonella enteritidis* phage types by plasmid profile typing

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## SUMMARY

Differentiation of *Salmonella enteritidis* by plasmid profile typing has been compared to differentiation by phage typing. Examination of the type strains of the 27 *S. enteritidis* phage types showed that only 11 profile patterns could be identified. Moreover, two profile patterns were found in 15 of the type strains, including those of the two most common phage types in Britain, types 4 and 8. On this basis, plasmid profile typing is not as sensitive as phage typing for the primary subdivision of *S. enteritidis*.

When differentiation of 534 strains of the 27 phage types was attempted using plasmid profiles, variation in pattern suitable for epidemiological subdivision was found in 13 phage types and there were 9 profile patterns in strains of phage type 4. Plasmid profile typing can, therefore, be regarded as an effective adjunct to phage typing for the subdivision of *S. enteritidis*.

## INTRODUCTION

In 1987, *Salmonella enteritidis* was the second most common serotype causing salmonella food-poisoning in England and Wales. The Division of Enteric Pathogens (DEP) received 6858 strains from humans and this represented a six-fold increase on the number received in 1981. This increase has continued during 1988 and *S. enteritidis* is now the most common serotype in humans in Britain (B. Rowe, unpublished observations).

Within the serotype, phage typing provides a rapid method of strain discrimination which is useful in support of epidemiological investigations. The *S. enteritidis* phage typing scheme developed in the DEP uses 10 phages, and 27 phage types (PT's) have been described (Ward *et al.* 1987). However, of 16092 strains received during the period 1981–6, four phage types, PT's 4, 8, 6 and 11 comprised 92% of strains and in particular, 85% of strains belonged to PT's 4 and 8 (Ward *et al.* 1987). Therefore for epidemiological surveillance studies, further subdivision of these two phage types is desirable.

Plasmid profile typing has been particularly useful in tracing epidemics caused by multiresistant phage types of *S. typhimurium* (Threlfall *et al.* 1987; Wray *et al.* 1987) and it has also been used to subdivide drug-sensitive phage types of this serotype (Brunner *et al.* 1983). Most strains of *S. enteritidis* are drug-sensitive and



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the purpose of the present study was to compare plasmid profile typing with phage typing for strain differentiation within this serotype. Initially the approach adopted was to determine the plasmid profile pattern of the first recorded isolations of each phage type, referred to as the type strains. The profile patterns and degree of profile variation within isolations of each phage type were then investigated, with particular reference to PT's 4 and 8, the two most common types in Britain from 1981 to 1987.

## MATERIALS AND METHODS

### *Bacterial strains*

Strains of *S. enteritidis* described in this study were referred to the DEP for phage typing by Public Health and hospital laboratories in England and Wales, by the Scottish Salmonella Reference Laboratory and by laboratories of the Veterinary Investigation Service. Phage typing was as described by Ward *et al.* (1987) and, whenever possible, at least two epidemiologically unrelated strains were selected from each of the 27 *S. enteritidis* phage types for plasmid profile analysis. Because of the influence of antibiotic resistance plasmids on both plasmid profile and phage type, only drug-sensitive strains were included unless otherwise stated.

### *Isolation of partly-purified plasmid DNA and agarose gel electrophoresis*

Partly-purified plasmid DNA was isolated by a modification of the method of Kado & Liu (1981), using 1·5 ml volumes of strains grown at 37 °C with gentle aeration for 18 h. After centrifugation and resuspension in 20 $\mu$l of 50 mM TrisCl/1 mM EDTA, pH 8·0, cells were lysed by incubation for 30 min at 55 °C with 0·05 M Tris/3% SDS at pH 12·54. Chromosomal DNA, protein/SDS complexes, RNA and cell wall debris were removed by extraction with TE-saturated phenol/chloroform and 50 $\mu$l of the resultant plasmid DNA solution was analysed by electrophoresis on vertical slab agarose gels (Threlfall *et al.* 1986). All samples were initially screened on gels containing 0·7% agarose (w/v, Sigma, Type II), for 2·5 h at 140v, and molecular weights (MWs) were determined in relation to the mobility of 4 reference plasmids with MWs ranging from 98·0 to 4·6 MDa, carried in *Escherichia coli* K12, strain 39R861 (Threlfall *et al.* 1986). Plasmid DNA from strains carrying plasmids of less than 5·0 MDa was then screened again on gels containing 1·0% agarose, using strain V517 (Macrina *et al.* 1978), as a molecular weight standard to obtain an accurate measurement of plasmids in the range 1·0–5·0 MDa. Gels containing 1% agarose were run at 150v for 3·5 h before staining for 30 min in distilled water containing 5 $\mu$g/ml of ethidium bromide.

## RESULTS

### *Plasmids in* S. enteritidis *type strains*

Plasmids were found in 25 of the 27 *S. enteritidis* type strains and 10 profile patterns (PP's) were identified (Table 1). The only type strains found to be plasmid-free were those of PT's 7 and 16. The profile pattern (PP) of plasmid-free strains was designated SE 0.

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Table 1. *Plasmids in the type strains of* Salmonella enteritidis *phage types*

| Phage type (PT) | MW of plasmid DNA (MDa) | | | | | | | | | | | Profile pattern (PP) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | — | — | — | 38 | — | — | — | — | — | — | — | SE38 |
| 2 | — | — | — | 38 | — | — | — | — | — | — | — | SE38 |
| 3 | — | — | 59 | — | — | — | — | — | — | — | — | SE59 |
| 4 | — | — | — | 38 | — | — | — | — | — | — | — | SE38 |
| 4a | 70 | — | — | 38 | — | — | — | — | — | — | — | SE38a |
| 5 | — | — | — | 38 | — | — | — | — | — | — | — | SE38 |
| 6 | 70 | — | — | 38 | — | — | — | — | — | — | — | SE38a |
| 6a | — | — | 59 | — | — | 4·0 | 3·8 | — | — | — | — | SE59a |
| 7 | — | — | — | — | · | — | — | — | — | — | — | SE 0 |
| 8 | — | — | — | 38 | — | — | — | — | — | — | — | SE38 |
| 9 | — | — | — | 38 | — | — | — | — | — | — | — | SE38 |
| 10 | — | 65 | — | — | — | — | — | — | — | — | — | SE65 |
| 11 | — | — | 59 | — | — | — | — | — | — | — | — | SE59 |
| 12 | — | — | — | 38 | — | — | — | — | — | — | — | SE38 |
| 13 | — | — | — | 38 | — | — | — | — | — | — | — | SE38 |
| 14 | — | — | 59 | — | — | — | — | — | — | — | — | SE59 |
| 15 | — | — | — | 38 | — | — | — | — | — | — | — | SE38 |
| 16 | — | — | — | — | · | — | — | — | — | — | — | SE 0 |
| 17 | — | — | — | 38 | — | — | — | — | — | — | — | SE38 |
| 18 | — | — | — | 38 | — | — | — | — | — | — | — | SE38 |
| 19† | — | — | 59 | — | — | 4·0 | — | — | — | — | — | SE59b |
| 20 | — | — | 59 | — | — | — | — | — | — | — | — | SE59 |
| 21 | — | — | — | 38 | — | — | — | 3·0 | 2·8 | — | — | SE38b |
| 22 | — | — | — | 38 | 34 | — | — | — | — | — | — | SE38c |
| 23 | — | — | — | 38 | — | — | — | — | — | — | — | SE38 |
| 24 | — | — | — | 38 | — | — | — | 3·0 | — | 2·0 | — | SE38d |
| 25 | — | — | — | 38 | — | 4·0 | 3·8 | — | — | — | 1·0 | SE38e |
| 27 PT's | | | | | | | | | | | | 11 PP's |

·, No plasmid DNA detected. †Ampicillin-resistant.

A plasmid of 38 MDa was found in 18 of the 25 plasmid-carrying type strains. In 12 of these strains the 38 MDa plasmid was the only plasmid species identified whilst in 6 strains the 38 MDa plasmid was found together with one or more additional plasmids of varying sizes. The profile pattern of strains carrying only the 38 MDa plasmid was designated SE38 and those of strains carrying additional plasmids, SE38a, SE38b, SE38c, SE38d and SE38e. PP SE38a, characterized by plasmids of 38 and 70 MDa, was found in two type strains, those of PT's 4a and 6 but the four patterns designated SE38b to SE38e were each found in only one type strain, those of PT's 21, 22, 24 and 25.

The next most common plasmid identified was of 59 MDa and this plasmid was found in six type strains. It occurred alone in four strains and together with additional plasmids of different sizes in two strains. The profile pattern of strains carrying only the 59 MDa plasmid was designated SE59, and those of strains carrying additional plasmids, SE59a and SE59b (Table 1). Finally, one type strain was characterized by the carriage of a single plasmid of approximately 65 MDa. This pattern was designated SE65.

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Table 2. *Plasmid profile patterns in 534 strains of* S. enteritidis

| MW of plasmid DNA (MDa) | | | | | | | | | | | | | | | | | | PP |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| — | — | — | — | — | — | — | — | — | — | · | — | — | — | — | — | — | — | SE 0 |
| — | — | — | — | — | 38 | — | — | — | — | — | — | — | — | — | — | — | — | SE38 |
| — | 70 | — | — | — | 38 | — | — | — | — | — | — | — | — | — | — | — | — | SE38a |
| — | — | — | — | — | 38 | — | — | — | — | — | — | — | — | 3·0 | 2·8 | — | — | SE38b |
| — | — | — | — | — | 38 | 34 | — | — | — | — | — | — | — | — | — | — | — | SE38c |
| — | — | — | — | — | 38 | — | — | — | — | — | — | — | — | 3·0 | — | — | 2·0 | SE38d |
| — | — | — | — | — | 38 | — | — | — | — | — | — | 4·0 | 3·8 | — | — | — | — | SE38e |
| — | — | — | — | — | 38 | — | — | — | — | — | — | — | — | — | 2·8 | 2·4 | — | SE38f |
| — | — | — | — | — | 38 | — | — | — | — | — | — | — | — | 3·0 | — | — | — | SE38g |
| — | 70 | — | — | — | 38 | — | — | — | 20 | — | — | — | — | — | — | — | — | SE38h |
| — | — | — | — | — | 38 | — | — | — | — | 4·8 | 4·6 | — | — | 3·0 | — | — | — | SE38i |
| — | — | — | — | — | 38 | — | — | — | — | — | — | — | — | — | — | — | — | SE38j |
| 80 | 70 | — | — | — | 38 | — | 30 | — | — | — | — | — | — | — | — | — | — | SE38k |
| — | — | — | — | — | 38 | — | — | 26 | — | — | — | — | — | — | — | — | — | SE38l |
| — | — | — | — | — | 38 | — | — | — | — | — | — | — | — | — | — | — | 2·0 | SE38m |
| — | — | — | — | — | 38 | 34 | — | — | — | — | 4·6 | — | 3·8 | — | — | — | — | SE38n |
| — | — | — | — | — | 38 | — | 30 | — | — | — | — | — | — | — | — | — | — | SE38o |
| — | — | — | 59 | — | — | — | — | — | — | — | — | — | — | — | — | — | — | SE59 |
| — | — | — | 59 | — | — | — | — | — | — | — | — | 4·0 | 3·8 | — | — | — | — | SE59a |
| — | — | — | 59 | — | — | — | — | — | — | — | — | 4·0 | — | — | — | — | — | SE59b |
| — | — | — | 59 | 40 | — | — | — | — | — | — | — | — | — | — | — | — | — | SE59c |
| — | — | 65 | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | SE65 |
| — | — | 65 | — | — | — | — | — | — | 20 | — | — | — | — | — | — | — | — | SE65a |
| | | | | | | | | | | | | | | | | | | 23 PP's |

*Distribution of profile patterns in* S. enteritidis *phage types*

A total of 534 strains of the 27 *S. enteritidis* phage types was plasmid-typed. At least two epidemiologically unrelated strains of each phage type were studied with the exception of PT19, because only one isolation of this phage type was detected during the period of study. The MW's of the plasmids identified in the strains and the corresponding profile pattern designations are shown in Table 2.

In all, 23 profile patterns were observed (Table 2). The most common pattern was that of SE38, found in 383 strains of 19 phage types (Table 3). The second most common pattern was that designated SE38f, found in 41 strains of PT4 and 4 strains of PT9, followed by SE59, found in 25 strains of 6 phage types.

In 10 of the 27 phage types, all the isolations had profile patterns indistinguishable from those of the corresponding phage type strain. However, in 17 phage types at least one isolate had a profile pattern which differed from that of the type strain and in eight phage types, PT's 1, 4, 4a, 6, 6a, 8, 10 and 15, three or more PP's were identified (Table 3). In PT7, the type strain was plasmid-free but the 38 MDa plasmid was identified in 19 of 20 further isolations. Likewise, the type strain of PT16 was plasmid-free but 3 of 4 other isolates carried the 38 MDa plasmid. Three strains of PT4, one strain of PT8 and one strain of PT14 were also plasmid-free. The type strain of PT19 was ampicillin-resistant and carried two plasmids of 59 and 4·0 MDa. Transformation experiments demonstrated that in this strain, ampicillin resistance was encoded by the 4·0 MDa plasmid. The other isolate of PT19 was ampicillin-sensitive and did not carry the 4·0 MDa plasmid; in all other respects this strain was identical to the type strain.

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Table 3. *Distribution of plasmid profile patterns in* S. enteritidis *phage types*

| | Plasmid profile pattern | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| PT | 0 | 38 | 38a | 38b | 38c | 38d | 38e | 38f | 38g | 38h | 38i | 38j | 38k | 38l | 38m | 38n | 38o | 59 | 59a | 59b† | 59c | 65 | 65a | Totals |
| 1 | — | 6* | — | — | — | — | — | — | 1 | — | 1 | — | — | — | — | — | — | — | — | — | — | — | — | 8 |
| 2 | — | 6* | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | 6 |
| 3 | — | — | — | — | — | — | — | — | — | — | — | — | — | 1 | — | — | — | 2* | — | — | — | — | — | 3 |
| 4 | 3 | 193* | 4 | — | 1 | — | — | 41 | 1 | 1 | 2 | 1 | — | — | — | — | — | — | — | — | — | — | — | 247 |
| 4a | — | 1 | 1* | — | — | — | — | — | 1 | — | — | — | 1 | — | 1 | — | — | — | — | — | — | — | — | 5 |
| 5 | — | 5* | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | 5 |
| 6 | — | 1 | 5* | — | — | — | — | — | — | — | 22 | — | — | — | — | — | — | — | — | — | — | — | — | 28 |
| 6a | — | — | 1 | — | — | — | — | — | — | — | — | — | — | — | — | 1 | — | — | 3* | — | — | — | — | 5 |
| 7 | 1* | 19 | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | 20 |
| 8 | 1 | 96* | — | — | — | — | — | — | 2 | — | — | — | — | — | — | — | — | — | — | — | — | — | — | 99 |
| 9 | — | 6* | — | — | — | — | — | 4 | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | 10 |
| 10 | — | 1 | — | — | — | — | — | — | — | — | — | — | — | 1 | — | — | — | — | — | — | — | 2* | — | 4 |
| 11 | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | 9* | — | — | 1 | — | — | 10 |
| 12 | — | 3* | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | 3 |
| 13 | — | 23* | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | 23 |
| 14 | 1 | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | 5* | — | — | — | — | — | 6 |
| 15 | — | 3* | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | 1 | — | — | — | — | 1 | 5 |
| 16 | 1* | 3 | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | 4 |
| 17 | — | 2* | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | 2 |
| 18 | — | 6* | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | 6 |
| 19† | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | 1 | — | — | — | — | — | 1 |
| 20 | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | 7* | — | — | — | — | — | 7 |
| 21 | — | — | — | 3* | — | — | — | — | — | — | — | — | — | — | — | — | 5 | — | — | — | — | — | — | 8 |
| 22 | — | — | — | — | 2* | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | 2 |
| 23 | — | 4* | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | 4 |
| 24 | — | 5 | — | — | — | 2* | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | 7 |
| 25 | — | — | — | — | — | — | 6* | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | 6 |
| Totals | 7 | 383 | 11 | 3 | 3 | 2 | 6 | 45 | 5 | 1 | 25 | 1 | 1 | 2 | 1 | 1 | 5 | 25 | 3 | 0 | 1 | 2 | 1 | 534 |

* Indicates profile pattern of type strain

† Profile pattern SE59b was seen only in the type strain of PT19.

*Profile patterns in PT's 4 and 8*

The most common *S. enteritidis* phage types in Britain from 1981 to 1987 were PT's 4 and 8. Nine profile patterns were observed in 427 strains of PT4. The two most common patterns were SE38 (78·1% of strains) and SE38f, a pattern characterized by plasmids of 38, 2·7 and 2·4 MDa (16·6% of strains). Plasmids of 70 and 38 MDa were identified in four strains (SE38a), 38, 4·8, 4·6 and 3·0 MDa in two strains (SE38i) and three strains were plasmid-free (SE 0). The remaining four PP's in PT4 (SE38c, SE38g, SE38h and SE38j) were each observed in only one isolate of this phage type.

In PT8, 97% of strains carried only the 38 MDa plasmid (SE38). Only two strains carried an additional plasmid which in each case was of 3·0 MDa. This profile pattern was designated SE38g. The remaining strain was plasmid-free.

## DISCUSSION

Various techniques have been used to subdivide salmonella serotypes. These include biotyping, phage typing, antibiotic sensitivity testing and plasmid characterization by genetical and physical methods. With *S. enteritidis*, phage typing has proved a highly successful method of strain discrimination and has been used extensively for epidemiological investigations. Plasmid profile typing has been suggested as an alternative to phage typing for *S. typhimurium* (Holmberg *et al.* 1984) and one of the purposes of the current investigation was to see whether this approach could be used for the subdivision of *S. enteritidis*. Eleven profile patterns were identified in the 27 *S. enteritidis* phage type strains of Ward *et al.* (1987). On this basis it is clear that plasmid profile typing is not as discriminatory as phage typing for the primary subdivision of this serotype. When the profile patterns are analysed further, the lack of discrimination becomes more evident since the type strains of 11 phage types all carried a single plasmid of 38 MDa and a further four types all carried a single plasmid of 59 MDa. Thus, 15 of the 27 type strains of *S. enteritidis* fall within two profile patterns, SE38 and SE59. Nine plasmid profiles were observed in the remaining 12 type strains and specific patterns were found in the type strains of only five phage types, PT's 6a, 10, 21, 24 and 25.

Examination of 534 isolations of different phage types again demonstrated the ubiquitous nature of the SE38 and SE59 profile patterns. The SE38 pattern was found in at least one isolation of 19 phage types and the SE59 pattern was seen in five phage types. The only plasmid profile specific to all strains of a particular phage type and not observed in strains of any other phage type was SE38e. This pattern, characterized by four plasmids of 38, 4·0, 3·8 and 1·0 MDa, was found in all of six apparently unrelated isolations of PT25.

Seven strains of five phage types were plasmid-free, as were the type strain of PT's 7 and 16. The 38 MDa and 59 MDa plasmids can be spontaneously last at low frequency ($< 10^{-6}$) from *S. enteritidis* strains (unpublished observations) and the plasmid-free strains have probably been derived by this method.

The results show that plasmid profile typing is not as discriminatory as phage typing for the primary subdivision of *S. enteritidis*. It may be useful to subdivide

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certain phage types and for strains currently isolated in Britain, it is particularly useful in PT4. Ideally, the method should be used as an adjunct to phage typing in support of epidemiological investigations. Nevertheless, plasmid profile typing may be a simple technique to provide limited strain differentiation for laboratories which do not have access to phage typing.

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