J. Hyg., Camb. (1986), 97, 193–197
Printed in Great Britain

Recognition of the cryptic plasmid, pSLT, by restriction fingerprinting and a study of its incidence in Scottish salmonella isolates

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(*Received 10 April 1986; accepted 6 June 1986*)

SUMMARY

The plasmid pSLT is a cryptic plasmid of 60 megadaltons (Md) present in *Salmonella typhimurium* LT2. We present evidence that it has a characteristic fingerprint when digested with the restriction enzymes *Pst I* and *Sma I*. Among a representative collection of *S. typhimurium* isolates it was present in 67 % of strains and was widely distributed amongst different phage types (DT) with the exception of DT10 and U285. Furthermore, its prevalence among veterinary isolates was significantly higher than among human isolates. It was not found among any of the 96 strains representative of other salmonella serotypes currently prevalent and thus appears to be serotype-specific.

INTRODUCTION

Serotype-specific plasmids have been recognized in *Salmonella dublin* (Baird, Manning & Jones, 1985), *S. enteritidis* (Nakamura *et al.* 1985) and *S. typhimurium* (Popoff *et al.* 1984). These plasmids are usually cryptic although they have been implicated in host pathogenicity (Terakado *et al.* 1983; Hemuth *et al.* 1985).

The plasmid pSLT was first recognized in *S. typhimurium* LT2 (Dowman & Meynell, 1970) and its molecular weight estimated as 60 Md, although no plasmid-specified function was indentified (Spratt, Rowbury & Meynell, 1973). Early work suggested that it encoded fi^+ activity and was related to F-like plasmids (Smith *et al.* 1973). Among strains of *S. typhimurium*, plasmids with molecular weights of 60 Md (90 kbp) were reported as quite common by Jones *et al.* (1982) who also suggested that they were similar to the plasmid pSLT (MP10) of *S. typhimurium* LT2 and were involved in adhesion and invasion of HeLa cells in *in vitro* models.

We have applied a restriction fingerprinting method recently developed in this laboratory to provide answers to the following questions. Are the 60 Md plasmids of *S. typhimurium* related to each other and to pSLT? Are they serotype-specific? What is their incidence in a representative sample of *S. typhimurium* isolates? Are they associated with both human and veterinary sources and furthermore are they equally distributed among the common phage types?

MATERIALS AND METHODS

Bacterial strains

Salmonella typhimurium LT2 (ATCC 235564) was kindly provided by Dr J. Coote (Department of Microbiology, University of Glasgow). Ninety-eight strains of *S. typhimurium* and 96 strains belonging to other salmonella serotypes were obtained from the Scottish Salmonella Reference Laboratory (SSRL). These had been referred to SSRL from centres throughout Scotland. Multiple isolates from a single source or from known epidemiological episodes and outbreaks were excluded from the collection, which comprised strains from human, veterinary and environmental sources.

All isolates were confirmed as salmonellas by the biochemical methods of Edwards & Ewing (1972), serotyped by the method of Kauffman (1972) and phage-typed according to the method of Callow (1959) as extended by Anderson (1964).

Plasmid analysis

Plasmid DNA preparation and restriction enzyme fingerprinting were carried out using a sequential strategy (Platt *et al.* 1986) and the enzymes used in this study were *PstI* and *SmaI* (Gibco-BRL, Paisley, Scotland). Digestion conditions were as recommended by the manufacturer and fragment sizes were calculated by calibration of each gel with bacteriophage lambda DNA digested with *PstI*.

Statistical analysis

The prevalence of pSLT in human and veterinary isolates was compared using a chi-squared test incorporating Yates correction for continuity (Siegel, 1956).

RESULTS

Identification of pSLT by electrophoresis of restriction endonuclease digest fragments

Plasmid DNA from *S. typhimurium* LT2 produced a characteristic fingerprint after digestion with *PstI* and *SmaI*. The fragmentation pattern of pSLT with these enzymes is shown in Fig 1, which also shows the characteristic fingerprint of plasmid DNA extracted from clinical strains and includes strains that harbour additional plasmids. Digestion with *PstI* resulted in 15 visible fragments (> 800 base pairs (bp) and < 14 kbp), whereas digestion with *SmaI* generated 25 fragments in the same size range. The molecular weight of pSLT was calculated by addition of the molecular weight of digestion fragments and gave an estimate of 57 Md (91 kbp).

By directly comparing the fingerprints of pSLT with the *PstI* and *SmaI* fragmentation patterns of human and veterinary salmonellas, it was possible to determine those strains that harboured plasmids indistinguishable from pSLT.

The prevalence of pSLT in strains of *S. typhimurium* is shown by phage type in Table 1, as is its prevalence in isolates of veterinary origin.

Of the 98 strains of *S. typhimurium* studied, pSLT was detected in 66 strains (67·4%) and was equally distributed between different phage types with the notable exception of DT10 and U285.

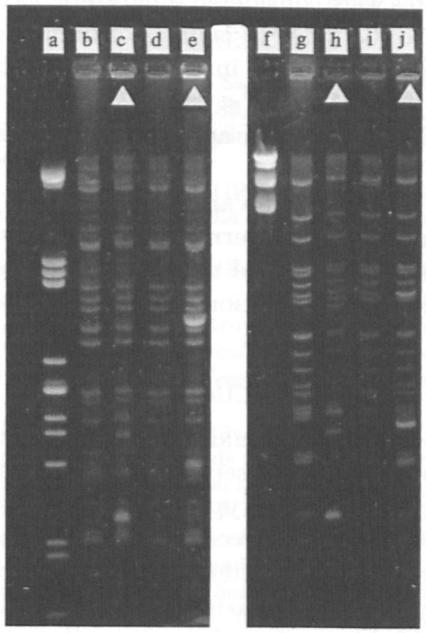

Fig. 1. *Pst I* and *Sma I* fingerprints, respectively, of plasmid DNA from *S. typhimurium*: LT2 (lanes b and g) and clinical strains (lanes c/h, d/i and e/j). Lanes a and f show fragments of bacteriophage Lambda DNA digested with *Pst I* and *Sma I* respectively. These lanes also show fragments derived from ▽ additional small plasmids.

Table 1. *Prevalence of the cryptic plasmid pSLT in* Salmonella typhimurium *(by phage type) and other serotypes determined by restriction fingerprinting*

S. typhimurium DT type	Number of isolates*	Number of isolates* containing pSLT
204c	11 (6)	11 (6)
49	11 (4)	10 (4)
U285	10	0
10	9	0
110	6 (2)	5 (1)
141	5 (3)	5 (3)
193	5 (3)	5 (3)
12	5 (2)	4 (1)
40	4 (2)	1
204	4 (2)	4 (2)
2	2 (2)	2 (2)
44	2 (1)	2 (1)
49a	2	2
99	2 (2)	2 (2)
104	2	2
U286	2 (2)	2 (2)
Others	16 (2)	9 (2)
Total	98 (33)	66 (29)
Other serotypes	96 (17)	0

* Veterinary isolates in parentheses.

https://doi.org/10.1017/S0022172400065268 Published online by Cambridge University Press

When veterinary isolates were considered separately, 29 of 33 (87·9%) contained pSLT compared with 37 of 65 (56·9%) isolates of human origin.

Comparison of the incidence of pSLT in human and veterinary isolates indicated a significant difference ($\chi^2 = 8{\cdot}1819$; $P < 0{\cdot}005$).

No evidence of pSLT was found in any of the 96 stains of other salmonella serotypes.

Fifteen strains of *S. typhimurium* of various phage types harboured pSLT as the only plasmid. The fragmentation pattern of the plasmid with both enzymes was identical in these strains, and indicated that there had been neither gain nor loss of restriction sites nor detectable deletions or insertions between them.

DISCUSSION

This study has shown that the plasmid pSLT is common among clinical strains of *S. typhimurium* in Scotland but is absent from other serotypes of salmonella, and strongly suggests that it is a serotype-specific plasmid. Since it is neither conjugative nor readily mobilized by conjugative R-plasmids (Spratt, Rowbury & Meynell, 1973), its prevalence indicates clonal dissemination and its association with diverse phage types of *S. typhimurium* further suggests clonal dissemination prior to the divergence of this serotype into currently recognized phagovars.

The high incidence and wide distribution, and the conservation of fragmentation pattern of this plasmid among different strains of *S. typhimurium* suggests that it must confer some advantage on the host. Although no plasmid function except fi^+ (Anderson & Smith, 1972) has been clearly established, these results support the findings of Helmuth *et al.* (1985) that pSLT is involved in pathogenicity.

The significant difference in the incidence of pSLT in human and in veterinary isolates is largely explained by the absence of the plasmid from DT10 and U285 isolates and the absence of veterinary isolates of these phage types. The absence of veterinary strains of these phage types from our collection reflects their current low incidence in Scotland. Although DT10 was relatively common among bovine isolates in Scotland during the period 1981-2 (Communicable Disease Surveillance Centre, unpublished) it has declined in recent years, and none was referred to SSRL during the period of our collection.

One implication of these results concerns the use of plasmid analysis in epidemiological studies. The demonstration that two (or more) bacterial strains harbour a single indistinguishable plasmid is often taken as evidence in support of local epidemiological linkage. However, this is based on the assumption that two strains are unlikely to have acquired the same plasmid by chance. The high incidence of pSLT among unrelated isolates of *S. typhimurium* indicates that such an assumption is clearly unjustified in situations where clonal dissemination contributes to the overall epidemiological process.

We are grateful to the Greater Glasgow Health Board Research Support Group for financial support.

REFERENCES

ANDERSON, E. S. (1964). The phage typing of Salmonellae other than *S. typhi*. In *The World Problem of Salmonellosis* (ed. E. van Oye), p.89, The Hague: Dr W. Junk.

ANDERSON, E. S. & SMITH, H. R. (1972). Fertility inhibition in strains of *Salmonella typhimurium*. *Molecular and General Genetics* **118**, 79–84.

BAIRD, G. D., MANNING, E. J. & JONES, P. W. (1985). Evidence for related virulence sequences in plasmids of *Salmonella dublin* and *Salmonella typhimurium*. *Journal of General Microbiology* **131**, 1815–1823.

CALLOW, B. R. (1959). A new phage typing scheme for *Salmonella typhimurium*. *Journal of Hygiene* **57**, 346–359.

DOWMAN, J. E. & MEYNELL, G. G. (1970). Pleiotropic effects of de-repressed bacterial sex factors on colicinogeny and cell wall structure. *Molecular and General Genetics* **109**, 57–68.

EDWARDS, P. R. & EWING, W. H. (1972). *Indentification of Enterobacteriaceae*. 3rd edn. Minneapolis, Minn: Burgess.

HELMUTH, R., STEPHAN, R., BUNGE, C., GOOD, B., STEINBECK, A. & BILLING, E. (1985). Epidemiology of virulence-associated plasmids and outer membrane protein patterns within seven common *Salmonella* serotypes. *Infection and Immunity* **48**, 175–182.

JONES, G. W., ROBERT, D. K., SVINARICH, D. M. & WHITFIELD, H. J. (1982), Association of adhesive, invasive and virulent phenotypes of *Salmonella typhimurium* with autonomous 60-megadalton plasmids. *Infection and Immunity* **38**, 476–486.

KAUFFMAN, F. (1972). *Serological Diagnosis of Salmonella Species*. Copenhagen: Munksgaard

NAKAMURA, M., SATO, S., PHYA, T., SUZUKI, S. & IKEDA, S. (1985). Possible relationship of a 36-magadalton *Salmonella enteritidis* plasmid to virulence in mice. *Infection and Immunity* **47**, 831–833.

PLATT, D. J., CHESHAM, J. S., BROWN, D. J., KRAFT, C. A. & TAGGART, J. (1986). Restriction enzyme fingerprinting of enterobacterial plasmids: a simple strategy with wide application. *Journal of Hygiene* **97**, 205–210.

POPOFF, M. Y., MIRAS, I., COYNAULT, C., LASSELIN, C. & PARDON, P. (1984). Molecular relationships between virulence plasmids of Salmonella serotypes typhimurium and dublin and large plasmids of other Salmonella serotypes. *Annales Microbiologie* (*Paris*) **135**. 389–398

SIEGEL, S. (1985) *Non-parametric Statistics for the Behavioural Sciences*. London: McGraw-Hill.

SMITH, H. R., HUMPHREYS, G. O., GRINDLEY. N. D. F., GRINDLEY, JUNE N. & ANDERSON, E. S. (1973). Molecular studies of an fi plasmid from strains of *Salmonella typhimurium*. *Molecular and General Genetics* **126**, 143–151.

SPRATT, B. G., ROWBURY, R. J. & MEYNELL, G. G. (1956). The plasmid of *Salmonella typhimurium* LT2. *Molecular and General Genetics* **121**, 347–353.

TERAKADO, N., SEKIZAKI, T., HASHIMOTO, K. & NAITOH, S. (1983). Correlation between the presence of a fifty-megadalton plasmid in *Salmonella dublin* and virulence for mice. *Infection and Immunity* **41**, 443–444.