J. Hyg., Camb (1986), 97, 199–204
Printed in Great Britain

The distribution of plasmids among a representative collection of Scottish strains of salmonellae

BY D. J. PLATT, D. J. BROWN

University Department of Bacteriology, Glasgow Royal Infirmary, Castle Street, Glasgow G4 0SF

AND D. S. MUNRO

Scottish Salmonella Reference Laboratory, Stobhill General Hospital, Glasgow G21 3UW

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

SUMMARY

The distribution of plasmids was studied in a representative collection of salmonella strains which comprised 98 *Salmonella typhimurium* and 96 other serotypes. Plasmids were detected in 72% of strains (mean 1·3 plasmids/strain) and individual strains harboured between 0 and 7 plasmids. They were more common among *S. typhimurium* than other serotypes (incidence 92 and 53%; mean 1·9 and 0·8 plasmids/strain respectively). Although a higher proportion of *S. typhimurium* (33%) were antibiotic-resistant compared to other serotypes (14%) the evidence presented indicated that R-plasmids were not responsible for the difference observed in the number and distribution of plasmids in these strains. These results were discussed in comparison with similar studies of *Escherichia coli* and other enteric genera.

INTRODUCTION

The contribution of plasmids to the clinical and economic problems associated with salmonellosis is well recognized. Some of the plasmids appear to be indigenous to the genus (Terakado *et al.* 1983; Nakamura *et al.* 1985), whereas others, notably those that confer resistance to antimicrobial agents, have been largely acquired by conjugation from other members of the enterobacteria (Datta, Richards & Datta, 1981; Platt, Sommerville & Gribben, 1984). Since, in addition to phenotypic modification by plasmids, the possession of plasmids confers considerable genetic flexibility upon the host strains (Platt *et al.* 1984), it can be argued that a clearer understanding of plasmid ecology and their behaviour in the natural environment requires the establishment of the relative abundance (and distribution) of plasmids among defined collections of the enteric genera. Here we report the distribution of plasmids among a representative collection of salmonellas and defined subgroups of strains and compare the results with those described for *Escherichia coli* (Platt *et al.* 1984) and other enteric genera (Platt, Chesham & Kristinsson, 1986).

MATERIALS AND METHODS

Bacterial strains

One hundred and ninety-four strains of salmonella were obtained from the Scottish Salmonella Reference Laboratory (SSRL). They had been referred to SSRL from centres throughout Scotland and the following criteria were observed in their selection. (1) Multiple isolates from a single source were excluded, as were multiple isolates from known epidemiological episodes and outbreaks on the basis of available epidemiological information; no attempt was made to determine historical relatedness. (2) The distribution of serotypes was chosen to reflect numerically current serotype prevalence in Scotland. The collection comprised strains from human, veterinary and environmental sources.

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

Standard methods were used for the growth and maintenance of cultures; DNA preparation and agarose gel electrophoresis have been previously described in detail (Platt & Sommerville, 1981; Platt *et al.* 1984).

Sensitivity testing

Sensitivity testing was carried out by disk diffusion, the radii of inhibition zones were recorded and compared with critical radii (R_c) obtained with *S. typhimurium* LT2 (ATCC 23564) for individual antimicrobial agents. Critical radii were calculated from $R_c = (\bar{x} - 3\sigma)$, where $\bar{x}$ was the mean radius obtained from 12 determinations and σ was the standard deviation. Any isolate that produced an inhibition zone for a given agent $< R_c$ was deemed to be resistant to that agent.

RESULTS

One hundred and ninety-four strains of salmonellas were examined. The distribution of serotypes is shown in Table 1, together with the phage types of 98 *S. typhimurium* strains which comprised half of the collection. After *S. typhimurium* the most common serotypes were *S. enteriditis* (9 %), *S. virchow* (5 %), *S. dublin* (5 %) and *S. saintpaul* (5 %). Resistance to antimicrobial agents was seen in 33 % of the *S. typhimurium* whereas among other serotypes it was less common (14 %). Multiple antibiotic resistance (three or more agents) among *S. typhimurium* was mainly found in phage types (DT) 204c and 49 with individual examples among DT 193 and 12. Among the other serotypes, 44 % of *S. saintpaul* and individual strains of *S. enteriditis* and *S. indiana* exhibited multiple resistance.

Plasmids were detected in 72 % of strains (mean 1·3 plasmids/strain) and individual strains harboured between 0 and 7 plasmids. However, there were notable differences between *S. typhimurium* and other serotypes. Ninety-two per cent of *S. typhimurium* possessed at least one plasmid (mean 1·9 plasmids/strain) whereas plasmids were found in only 53 % of combined other serotypes (mean 0·8 plasmids/strain). When antibiotic-resistant *S. typhimurium* were excluded and plasmids considered in the sensitive subset, 88 % of these strains harboured plasmids (mean 1·3 plasmids/strain). This indicates that differences in the preva-

Table 1 *The distribution of serotypes among 194 strains of salmonella and phage types among 98 strains of* S. typhimurium

Serotype	Number*	Phage type	Number*
S. typhimurium	98 (36)	2	2 (0)
		10	9 (0)
		12	5 (1)
		12a	1 (0)
		40	4 (0)
		44	2 (1)
		49	11 (5)
		49a	2 (1)
		99	2 (0)
		104	2 (0)
		104b	1 (0)
		110	6 (0)
		141	5 (0)
		193	5 (4)
		204	4 (4)
		204a	1 (1)
		204c	11 (11)
		U285	10 (0)
		Others	15 (8)
S. enteriditis	17 (2)		
S. dublin	9 (0)		
S. virchow	9 (0)		
S. saintpaul	9 (4)		
S. heidelberg	7 (2)		
S. panama	6 (0)		
S. infantis	6 (1)		
S. agona	5 (1)		
S. anatum	5 (0)		
S. derby	5 (1)		
S. indiana	5 (1)		
S. livingstone	5 (0)		
S. stanley	5 (0)		
S. newport	3 (1)		

* (Number resistant to at least one antibiotic.)

lence of plasmids among *S. typhimurium* compared to other serotypes are not related to the higher incidence of resistance in this serotype and by inference to the contribution of R-plasmids to the total plasmid complement. The frequency distributions of plasmids in the total collection and subpopulations are shown in Figure 1 (A–D).

In this study there were insufficient representatives of individual serotypes to consider the frequency distribution by serotype and it is therefore possible that one or more individual serotypes may harbour a similar number of plasmids to *S. typhimurium*. When the plasmids detected were considered in relation to phage type – for *S. typhimurium* – and serotype – for serotypes other than *S. typhimurium* – the following observations were notable: among *S. typhimurium*, a 60 Md plasmid was present in 67 % of isolates and all isolates of U285 harboured a plasmid of 2·1 Md. Seventy-six per cent of *S. enteriditis* possessed a plasmid of 36 Md and in all isolates of *S. dublin* a 50 Md plasmid was detected.

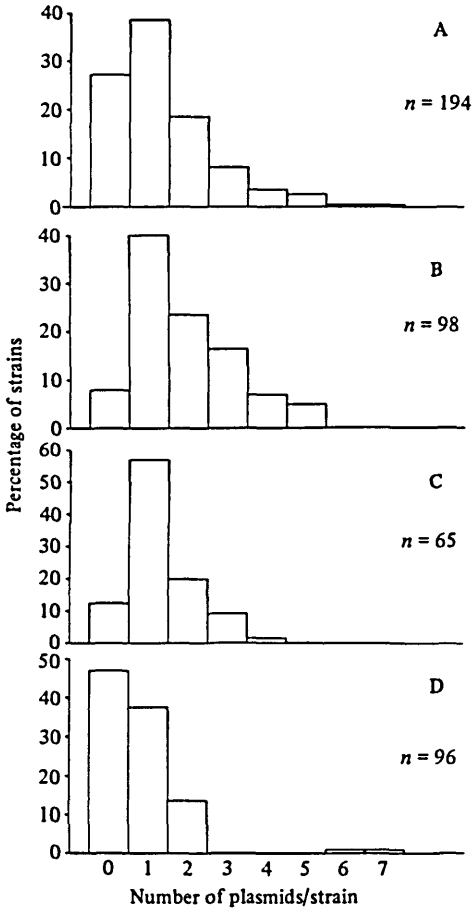

Fig. 1. The distribution of plasmids among: (A) 198 strains of salmonella; (B) the subpopulation of *S. typhimurium*; (C) the subpopulation of *S. typhimurium* after the exclusion of antibiotic-resistant strains; and (D) the combined other serotypes.

DISCUSSION

The results presented indicate that plasmids are common among strains of salmonella and the plasmids of similar molecular weight within individual serotypes may correspond to the serotype-specific plasmids demonstrated by Helmuth *et al.* (1985). The majority of plasmids are not associated with antibiotic resistance, although from the comparison of Fig. 1B with 1C resistant *S. typhimurium* clearly harbour more plasmids than sensitive strains, although the distribution in each case is similar. In previous studies (Platt *et al.* 1984; Platt *et al.* 1986) we have considered the distribution of plasmids in *E. coli*, various subpopulations thereof and other enteric genera. *E. coli* harboured a large number of plasmids (mean 1·8 plasmids/isolate), whereas plasmids were more sparsely found among the other enteric genera (mean 0·9 plasmids/isolate). In comparison the salmonellas appear to be intermediate (mean 1·3 plasmids/isolate) and furthermore, the relationship between *S. typhimurium* and other serotypes appears to be similar if not analogous to that between *E. coli* and other enteric genera. We previously suggested (Platt *et al.* 1986) that the ability to acquire and/or maintain plasmids may be related to genus, and a statistical model (Platt, submitted for publication) supports this view. A similar mechanism may be responsible for the difference in the prevalence of plasmids between *S. typhimurium* and other serotypes.

Comparison between the results described here for *S. typhimurium* and *E. coli* (Platt *et al.* 1986) shows remarkable similarities both in the mean number of plasmids (1·9 and 1·8/isolate respectively) and in their distribution.

Among the salmonellas, clonal dissemination has been shown to influence the prevalence of drug-resistant epidemic strains (Threlfall, Ward & Rowe, 1978; Casalino *et al.* 1984), and the demonstration of serotype-specific plasmids (Terakado *et al.* 1983; Nakamura *et al.* 1985; Brown, Munro & Platt, 1986) further suggests that this process operates on strains that harbour plasmids not associated with drug resistance. Clonal dissemination will clearly effect the distribution of plasmids, and thus it is possible that the similarity between *E. coli* and *S. typhimurium* is superficial, since representative collections of *E. coli* appear to contain few strains that harbour common plasmids as a result of clonal dissemination (Chesham & Platt, in preparation). The development of a quantitative approach towards the assessment of plasmid diversity in defined populations would clarify this situation.

We acknowledge 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.

BROWN, D. J., MUNRO, D. S. & PLATT, D. J. (1986). Recognition of the cryptic plasmid, pSLT, by restriction fingerprinting and a study of its incidence in Scottish salmonella isolates. *Journal of Hygiene* 97, 193–197.

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

CASALINO, M., COMANDUCCI, A., NICOLETTI, M. & MAIMONE, F. (1984). Stability of plasmid content in *Salmonella wien* in late phases of the epidemic history. *Antimicrobial Agents and Chemotherapy* 25, 499–501.

DATTA, N., RICHARDS, H. & DATTA, C. (1981). *Salmonella typhi in vivo* acquires resistance to both chloramphenicol and cotrimoxazole. *Lancet* i, 1181–1183.

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

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

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

NAKAMURA, M., SATO, S., OHYA, T., SUZUKI, S. & IKEDA, S. (1985). Possible relationship of a 36-megadalton *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.

PLATT, D. J., CHESHAM, J. S. & KRISTINSSON, K. G. (1986). R-plasmid transfer *in vivo*: a prospective study. *Journal of Medical Microbiology* **21**, 325–330.

PLATT, D. J. & SOMMERVILLE, J. S. (1981). A simple method for the detection of resistance plasmids in *Serratia* species by transfer to the genus *Enterobacter*. *Journal of Antimicrobial Chemotherapy* **8**, 145–152.

PLATT, D. J., SOMMERVILLE, J. S. & GRIBBEN, J. (1984). Sequential acquisition of R-plasmids *in vivo* by *Salmonella typhimurium*. *Journal of Antimicrobial Therapy* **13**, 65–69.

PLATT, D. J., SOMMERVILLE, J. S., KRAFT, C. A. & TIMBURY, M. C. (1984). Antimicrobial resistance and the ecology of *E. coli* plasmids. *Journal of Hygiene* **93**, 181–188.

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.

THRELFALL, E. J., WARD, L. R. & ROWE, B. (1978). Spread of multi-resistant strains of *Salmonella typhimurium* phage types 204 and 193 in Britain. *British Medical Journal* ii, 997.