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Restriction enzyme fingerprinting of trimethoprim resistance plasmids

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SUMMARY

Restriction enzyme fingerprinting was applied to 72 transferable trimethoprim resistance plasmids to examine aspects of their epidemiology and molecular relatedness.

These plasmids had previously been divided into 25 groups according to differences in mol. wts and in antimicrobial resistance determinants. Restriction enzyme fingerprinting allowed the plasmids to be further divided into 44 different groups. The groups based on molecular weight and resistance patterns often, but not invariably, corresponded with those based on restriction enzyme fingerprints. Some plasmids with the same mol. wt and resistance pattern had different digest fingerprints and conversely, although more rarely, plasmids which differed in molecular weight by as much as 10 MDa or in resistance pattern by one resistance marker, had indistinguishable fingerprints.

The plasmids were initially divided into three broad categories according to which restriction enzymes gave fingerprints of 6–20 fragments. These categories differed in the molecular weights of the plasmids contained, the numbers of resistance markers, and the proportions of the plasmids which carried transposon Tn7.

Some plasmids were more widespread and persistent than others with the same mol. wt and resistance pattern but with a different restriction enzyme fingerprint.

Thus, application of this technique has shown the trimethoprim resistance plasmids studied to be more diverse than was indicated by determination of mol. wt and resistance pattern, and has indicated changes in the plasmid pool over the 3 years during which they were collected.

INTRODUCTION

The first report of a transferable plasmid which mediated resistance to trimethoprim was published by Fleming, Datta & Gruneberg (1972). Trimethoprim resistance has since been found on plasmids of an increasing number of different incompatibility groups (Richards & Nugent, 1979).

In the course of earlier studies, we isolated a large number of plasmids that specified trimethoprim resistance (Kraft, Platt & Timbury, 1983; 1984; 1985) many of which carried transposon Tn7 (Kraft, Timbury & Platt, 1986). These

plasmids were originally grouped on the basis of molecular weight and resistance pattern.

Although incompatibility grouping (Datta, 1977) provides the means to further characterize plasmids, it is based on a variable and often small proportion of the plasmid DNA. It permits plasmids to be allocated to one of a limited number of groups but does not resolve small differences between related plasmids.

Therefore, to investigate further our collection of trimethoprim R-plasmids and examine aspects of their epidemiology, we used restriction endonuclease analysis of plasmid DNA, a method that has become widely used for the study of plasmid and bacterial epidemiology (Rubens *et al.* 1981; O'Brien *et al.* 1982; Lyon *et al.* 1984; Beul *et al.* 1985; Platt, Chesham & Kristinsson, 1986). In this study, we applied a simple fingerprinting strategy to investigate the similarities and differences between trimethoprim resistance plasmids.

MATERIALS AND METHODS

Plasmids. Seventy-two conjugative plasmids coding for high level resistance to trimethoprim (MIC > 1024 μg/ml) were studied. They were originally detected in clinical isolates of *Escherichia coli* and had been transferred to *E. coli* K12 strains J62-2 or J53-1 in the course of earlier studies (Kraft, Platt & Timbury, 1983; 1984; 1985). The clinical isolates had been collected on the basis of trimethoprim resistance from both hospitalized and domiciliary patients with urinary tract infections. Multiple isolates from the same patient were excluded from the study. The plasmids specified resistance to between one and seven antibiotics: trimethoprim (Tp), sulphamethoxazole (Su), streptomycin (Sm), tetracycline (Tc), ampicillin (Ap), chloramphenicol (Cm) and kanamycin (Km). Thirty-two of them had been shown to carry Tn7 (Kraft, Timbury & Platt, 1986).

Media. Cystine-lactose-electrolyte deficient (CLED) agar (Mast) was used for growth and maintenance of organisms. Brain heart infusion broth (BHI, Oxoid) was used for growth of organisms for preparation of plasmid DNA.

Preparation of plasmid DNA and restriction enzyme analysis. Organisms were grown in BHI at 37 °C overnight. Plasmid DNA was extracted using the alkaline–SDS method of Birnboim & Doly (1979) as modified by Ish-Horowitz & Burke (1981). In addition the preparations were treated with 7·5 M ammonium acetate before ethanol precipitation.

The plasmid DNA was digested with restriction enzymes according to the manufacturer's instructions. Reaction mixtures contained 20 μl plasmid DNA and 10 units of enzyme in a total volume of 50 μl. Incubation was for 4 h at the temperature recommended by the manufacturer for each individual enzyme.

Gel electrophoretic analysis of the digest fragments was carried out as described previously (Kraft, Timbury & Platt, 1986). Bacteriophage lambda DNA, digested with an appropriate enzyme, was included on each gel to provide molecular weight standards.

Restriction enzymes used were: *Hind* III, *Pst* I, *Sma* I, *Eco* RI and *Ava* II (BRL) and *Bsp* 1286 (New England Biolabs).

A 'fingerprint' of each plasmid was obtained with each of two or more restriction enzymes in order to obtain a fingerprint which consisted of 6–20 fragments (the

Table 1. *Transferable trimethoprim resistance plasmids*

Antimicrobial resistance pattern transferred	Mean plasmid mol. wt. (MDa)	Number of donor isolates that transferred plasmid		
		Hospital collection I (1979–80)	Hospital collection II (1982)	Collection III (Community)
Tp	46	0	1	0
Tp	68	0	0	1
TpSm*	35	3	2	2
TpSm†	47	8	0	1
TpSm‡	60	2	1	0
TpSm	73	1	0	0
TpTc‡	32	2	3	0
TpTc	86	0	1	0
TpKm	42	0	1	0
TpKm*	65	3	2	3
TpSmTc	48	3	0	0
TpSmAp	49	0	1	0
TpSmKm	74	1	0	0
TpSuSmTc	31	1	0	0
TpSuSmAp*	50	9	5	1
TpSmTcAp	62	0	1	0
TpTcApKm	66	0	1	0
TpTcApCm	55	0	0	1
TpSuSmTcAp	46	3	0	0
TpSuSmTcAp	67	1	0	0
TpSuSmCmKm	60	0	0	1
TpTcApCmKm	65	0	3	0
TpSuSmTcApCm	62	0	1	0
TpSuSmTcApCm	92	0	1	0
TpSuSmTcApCmKm	56	0	1	0

* Similar plasmid found in all three collections.
† Similar plasmid found in community and one hospital collection.
‡ Similar plasmid found in both hospital collections.

most suitable number for gel electrophoretic analysis). The enzymes were selected according to the strategy developed in our department (Platt *et al.* 1986). *Hind* III digestion had been carried out previously (Kraft, Timbury & Platt, 1986) and was included where appropriate.

RESULTS

The 72 plasmids were originally separated into 25 groups distinguishable by mol. wt and resistance pattern (Table 1).

On the basis of restriction enzyme analysis the plasmids could be divided into three broad categories according to which enzymes produced a 'fingerprint' of 6–20 fragments. Within each category, distinct groups were recognized by their fingerprints. The groups, based on mol. wt and resistance pattern frequently corresponded with those based on restriction enzyme fingerprints but this was not invariably the case. According to the fingerprints a total of 44 apparently different plasmids were distinguished.

Category 1. This category contained 26 plasmids which ranged in mol. wt from

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Table 2. *Restriction endonuclease analysis of trimethoprim R-plasmids.*

	Category 1 plasmids			*Bsp* 1286 fragments		
					No. common	
Group	Resistance pattern	Tn7	Plasmid mol. wt. (MDa)	No.	Within group	With other group
1	TpTc	—	37	9		
	TpTc	—	30	9		
	TpTc	—	30	9	9	
	TpTc	—	33	9		
	TpTc	—	34	9		
	TpSmTc	—	48	9		
1a	TpSuSmTc	—	31	9		8 (1)*
2	TpSmTc	+	52	17		
	TpSmTc	+	52	17		
	TpSm	+	52	17	17	
	TpSm	+	52	17		
	TpSm	+	59	17		
2a	TpSm	+	48	17		
	TpSm	+	46	17	17	13 (2)*
	TpSm	+	48	17		
2b	TpSm	+	52	16		9 (2)*
3	TpSm	+	46	7	7	
	TpSm	+	49	7		
4	TpSm	+	46	12	12	
	TpSm	+	36	12		
5	TpSm	+	36	12		
6	TpSm	+	36	12		
7	TpSm	+	34	5	2–3	
8	TpSm	+	30	12		
9	TpSm	+	33	16		
10	TpSm	—	36	11		

* Number of group.

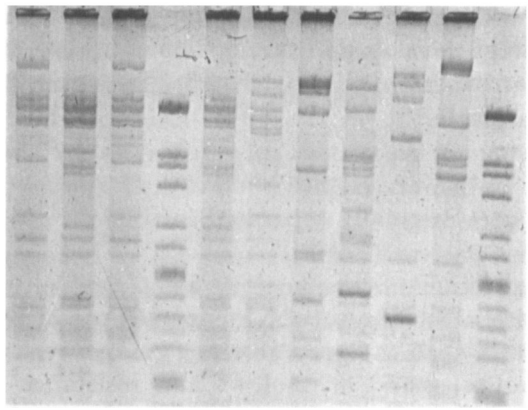

Fig. 1. Restriction endonuclease digests of TpSm plasmids with *Bsp* 1286. Category 1. From left to right: plasmids from GRI 20079 (group 2a), GRI 18579 (group 2), GRI 16679 (group 2a), Lambda standard (range 5·2–1·0 kb), GRI 16379 (group 2), GRI 15979 (group 2b), GRI 3483 (group 9), GRI 2183 (group 8), GRI 40982 (group 7), GRI 15482 (group 4), Lambda standard.

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Table 3. *Restriction endonuclease analysis of trimethoprim R-plasmids.*

	Category 2 plasmids			*Pst* I fragments		
					No. common	
Group	Resistance pattern	Tn7	Plasmid mol. wt. (MDa)	No.	Within group	With other group
1	TpKm	—	70	20		
	TpKm	—	70	20	20	
	TpKm	—	64	20		
1a	TpKm	—	69	20		18 (1)*
1b	TpKm	—	63	20		18 (1)*
1c	TpKm	—	70	19		19 (1)*
2	TpKm	—	42	18		9 (1)*
2a	TpKm	—	56	19		6 (2)*
2b	TpKm	—	66	23		7 (2)*
3	TpSm	+	60	19	16	
	TpSm	+	64	19		
4	TpTcApCmKm	—	67	15		
	TpTcApCmKm	—	65	15	15	
	TpTcApCmKm	—	64	15		
5	TpSmTcAp	—	62	17		
6	TpTcApCm	—	55	17		
7	TpTcApKm	—	66	20		
8	TpSuSmTcApCm	+	67	16		
9	TpSuSmCmKm	—	60	12		
10	TpSuSmTcApCm	—	62	20		
11	TpSuSmTcApCm	—	92	29	2–3	12 (10)*
12	TpSuSmTcApCmKm	—	56	16		
13	Tp	—	46	12		
14	Tp	—	68	15		9 13)*
15	TpTc	—	86	21		
16	TpSmAp	+	49	18		
17	TpSmKm	—	74	8		
18	TpSm	—	73	16		

* No. of group.

30–59 MDa and specified resistance to TpTc, TpSm, TpSmTc and TpSuSmTc. Eighteen carried Tn7.

On the basis of restriction enzyme fingerprinting they could be divided into 5 broad groups and subdivided further into 14 different plasmids 10 of which carried Tn7 (Table 2) (Fig. 1).

Group 1 contained one plasmid with an additional resistance marker and a larger mol. wt than the remaining plasmids although all produced the same fingerprint with *Bsp* 1286. Similarly, group 2 contained plasmids of two different resistance patterns but similar molecular weights and identical fingerprints with *Bsp* 1286. The other groups contained fewer plasmids and here identical fingerprints corresponded with identity of resistance pattern and similar mol. wt. Groups 5–10 consisted of 6 individual plasmids of similar mol. wt. and resistance pattern each with a different fingerprint and no more than 2 or 3 bands common to any 2 plasmids.

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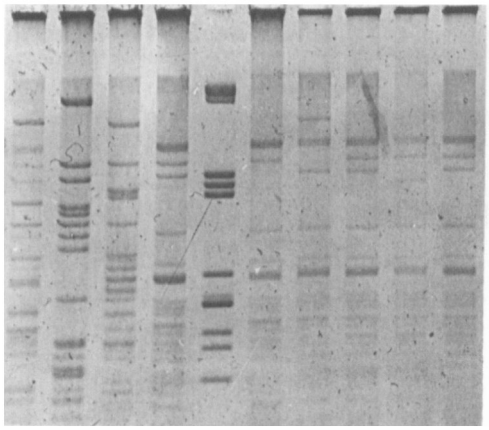

Fig. 2. Restriction endonuclease digests of TpKm plasmids with *Pst* I. Category 2. From left to right: plasmids from GRI 20882 (group 2), GRI 1480 (group 2a), GRI 2580 (group 2b), GRI 7480 (group 1), Lambda standard (range 14·5–1·7 kb), GRI 6180 (group 1c), GRI 4282a (group 1a), GRI 4282 (group 1), GRI 36282 (group 1b), GRI 52482 (group 1).

Table 4. *Restriction endonuclease analysis of trimethoprim R-plasmids.*

	Category 3 plasmids			*Hind* III fragments		
					No. common	
Group	Resistance pattern	Tn7	Plasmid mol. wt. (MDa)	No.	Within group	With other group
1	TpSuSmAp	+	46	16		
	TpSuSmAp	+	53	16		
	TpSuSmAp	+	47	16	16	12 (1)*
	TpSuSmAp	+	50	16		
	TpSuSmAp	+	50	16		
1a	TpSuSmAp	—†	49	16		
	TpSuSmAp	—†	48	16		
	TpSuSmAp	—†	53	16		
	TpSuSmAp	—†	55	16	16	
	TpSuSmAp	—†	48	16		
	TpSuSmAp	—†	50	16		
1b	TpSuSmAp	+	39	19		8 (1)* 11 (1a)*
1c	TpSuSmAp	+	46	18	bands not clear: matching uncertain	
1d	TpSuSmAp	—†	55	18		
1e	TpSuSmAp	—	50	?		
2	TpSuSmTcAp	+	41	17		
	TpSuSmTcAp	+	48	17	17	
2a	TpSuSmTcAp	+	50	17		16 (2)*

* No. of group.
† Smaller Tn7 fragment present.

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Further analysis of plasmids in this category was not achieved due to the limited number of cleavage sites.

Category 2. This category contained 28 plasmids only 4 of which carried Tn7. Mol. wts ranged from 42–92 MDa, 23 (82%) of the plasmids having a mol. wt of 60 MDa or greater, and 11 (39%) specified resistance to between 4 and 7 antibiotics. The commonest plasmid in this category specified resistance to TpKm. This category could be subdivided into 23 apparently different plasmids (Table 3). The TpKm plasmids in particular appeared to exhibit some diversity although the various groups differed by relatively few fragments (Fig. 2).

Category 3. All the 18 plasmids in category 3 coded for TpSuSmAp or TpSuSmTcAp with mol. wts ranging from 39–55 MDa. Ten carried Tn7. Eight different plasmids were distinguishable (Table 4), although the six plasmids in group 1 had a large proportion of fragments in common and those in group 2 differed by a single fragment only.

Relationship between grouping of plasmids by mol. wt and resistance pattern and by restriction digest patterns. The two plasmids that specified trimethoprim resistance only and differed in mol. wt by about 10 MDa both fell into category 2. Digestion with *Pst* I produced 12 and 15 fragments respectively of which 9 were common, suggesting a high degree of similarity.

The plasmids that specified resistance to TpSm, all except one containing Tn7, exhibited considerable heterogeneity, some falling into category 1, and others into category 2. The 7 TpSm plasmids with mol. wts of 30–36 MDa all gave distinct fingerprints with *Bsp* 1286 with only 2 or 3 common bands (Table 2). One of this group gave the same fingerprint as a TpSm plasmid 10 MDa larger. The 2 remaining mol. wt groups of TpSm plasmids in Category 1 were divided into 4 apparently different plasmids. This is consistent with the wide dissemination of Tn7 among different plasmids.

Of the 6 TpTc plasmids, 5 of similar mol. wt all gave the same fingerprint in category 1 whereas the larger TpTc plasmid fell into category 2. This indicated a common origin for the lower mol. wt. TpTc plasmids rather than the spread of a transposable element, although a larger number of plasmids would need to be analysed to confirm this. Three of these five plasmids came from organisms isolated from different patients in the same hospital ward during the same month, and the clinical isolates were indistinguishable on the basis of resistance pattern and plasmid profile. This was taken to indicate bacterial cross-infection.

The TpSmTc and TpSuSmTc plasmids were similar in molecular weight and fingerprints to TpSm and TpTc plasmids respectively, suggesting a common origin.

All the TpKm plasmids fell into category 2 and although they exhibited some heterogeneity in mol. wt and fingerprints, many differed by only 1 or 2 fragments (out of a total of 18–20) and at least one-third of the fragments were common to all. This again suggests a common origin rather than spread of a transposable element but indicates some plasmid divergence. The bacterial isolates from which these plasmids originally came were all apparently different on the basis of antimicrobial resistance patterns and plasmid profiles.

The plasmids with large numbers of resistance markers all tended to have

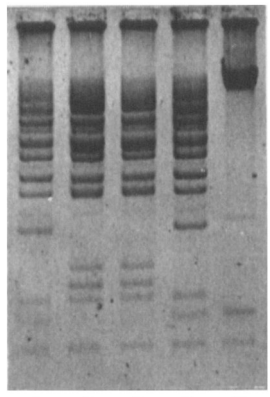

Fig. 3. Restriction endonuclease digests of plasmids coding for TpSuSmAp, with *Hind* III. Category 3. Lanes A and D, plasmids from isolates GRI 3479 and 22779 respectively, group 1. Each has the two fragments characteristic of transposon Tn7. Lanes B and C, 11779 and 17279 respectively, group 1a. The larger of the two fragments characteristic of Tn7 is not present. Lane E: Rp4::Tn7 standard plasmid.

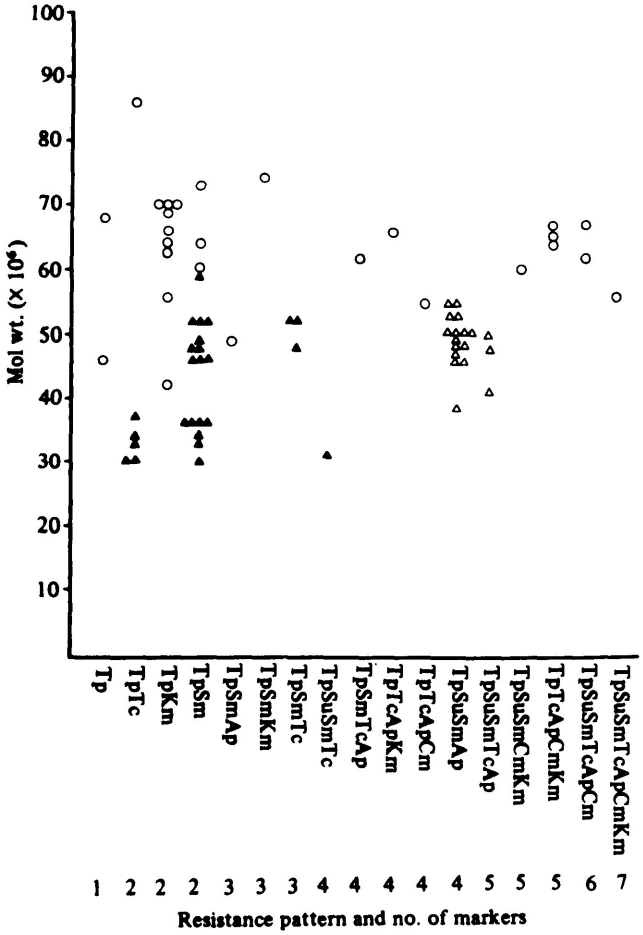

Fig. 4. Scatter diagram of molecular weights and resistance patterns of trimethoprim resistance plasmids divided into three categories on the basis of which restriction enzymes gave 6–20 digest fragments. ▲, category 1; ○, category 2; △, category 3.

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relatively high molecular weights and fell into category 2 although they had few fragments in common.

The plasmids that specified resistance to TpSuSmAp could be divided (with four exceptions) into two groups each group with a distinct fingerprint (Fig. 3). Group 1 (Table 4) carried Tn7 while group 1a yielded the same number of *Hind* III digest fragments as group 1, but whereas the smaller of the two Tn7 fragments was present the upper, larger, band was not; the two groups shared 12 common fragments (Fig. 3). This suggests a rearrangement involving part of the Tn7. The relationship of the remaining TpSuSmAp plasmids to the others is uncertain. Three repeatedly failed to produce a clear fingerprint although no reason for this was apparent. This problem may be related to the characteristics of the individual plasmids.

The relationship between mol. wt., resistance pattern and restriction digest category is illustrated in Fig. 4. Category 1 tended to have a small number of resistance markers and relatively low mol. wts., category 2 a wide range of resistance markers including all those with larger numbers of markers and relatively high mol. wts., and category 3 a very restricted group of plasmids with 4 or 5 resistance markers and intermediate mol. wt. approximately 50 MDa. Plasmids that encoded Tn7 were largely restricted to categories 1 and 3.

Epidemiology. The digest fingerprints were used to examine the similarity of plasmids from the three collections of organisms.

The plasmids coding for TpSm, overall the commonest resistance pattern isolated, are detailed in Table 5. TpSm plasmids were originally divided into four groups on the basis of molecular weight. It is clear from Table 5 that the incidence of the different TpSm plasmids in the three collections is related to the fingerprint category and group. Category 1 plasmids, mean mol. wt. 49 MDa (Set B) belonging to groups 2, 2a, 2b and 3, appeared only in the 1979 hospital collection. The one plasmid of mol. wt. 46 MDa from the community was isolated in 1981 and was in a different group (group 4). The miscellaneous group of low mol. wt. plasmids (Set A) persisted through all three collections. A category 2 group 3 plasmid (Set C) also persisted into the second hospital collection.

Considering the TpSuSmAp plasmids (category 3) – the second most common resistance pattern, of those with one Tn7 band missing (groups 1a and 1d) the 6 in group 1a were isolated in 1979 and the 1 in group 1d in 1982. In contrast, of the 7 plasmids with both Tn7 fragments, groups 1, 1b and 1c, 2 were isolated in 1979, 1 in 1980 and 4 in 1982.

DISCUSSION

From the results presented, it appears that the separation of the plasmids into three categories on the basis of restriction enzyme digestion reflects genuine differences in the plasmids as the categories also relate to the other plasmid characteristics of mol. wt. and antimicrobial resistance determinants.

The results have shown on the one hand, that plasmids with the same mol. wt and resistance pattern could have different fingerprints; thus increasing our estimate of the number of different trimethoprim R-plasmids. Conversely, plasmids

Table 5. *Restriction enzyme digests of TpSm plasmids isolated at different times*

Set	Collection: Hospital (1979–80) Mol. wt. (MDa)	Category	Group	Tn7	Hospital (1982) Mol. wt. (MDa)	Category	Group	Tn7	Community (1981–83) Mol. wt. (MDa)	Category	Group	Tn7
A	36	1	5	+	36	1	4	+	33	1	9	+
	36	1	6	+ +	30	1	8	+	36	1	10	—
	34	1	7	+								
B	52	1	2b	+	None				46	1	4	+
	52	1	2	+ +								
	48	1	2a	+ +								
	52	1	2	+ +								
	46	1	2a	+ +								
	48	1	2a	+ +								
	46	1	3	+ +								
	49	1	3	+								
C	59	1	2	+	64	2	3	+				
	60	2	3	+								
D	73	2	18	—								

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differing slightly in mol. wt. (by up to 10 MDa) or resistance pattern (one determinant) could give the same fingerprint. To remain undetected, the DNA responsible for an increase in mol. wt must be present in fragments sufficiently small or large to fall outside the mol. wt range of the fragments making up the fingerprint. In the case of the presence or absence of an extra resistance marker this could result from an inversion or rearrangement within a fragment not affecting the recognition sequences for a given enzyme. Alternatively, the additional DNA may be present in a sufficiently large fragment that the change in mol. wt is not resolved. Thus clearly the two types of characteristics need to be used in conjunction.

Heterogeneity, with few common fragments, among the fingerprints of TpSm plasmids suggests the continued spread of Tn7 through the plasmid pool. Fingerprints of TpKm and TpTc plasmids on the other hand do not support our earlier suggestion (Kraft, Platt & Timbury, 1984) that other trimethoprim transposons may be spreading through the plasmid pool. The similarity of these plasmids suggests that each may have had a common origin rather than resulting from the spread of transposons, and in the case of TpTc plasmids, bacterial cross-infection appears to have occurred. However, these results do not exclude the possibility that a transposon is responsible for TpKm resistance.

In the present study the fingerprints were also found to have some epidemiological applications. TpSm plasmids were less common in the later two collections than in the first and one particular mol. wt. group had disappeared altogether. The question arose as to whether this was a particular plasmid type or whether the finding was coincidental. Fingerprint results showed that the plasmids which had disappeared belonged to different groups to those which persisted and suggests a genuine change in the plasmid pool.

In the case of TpSuSmTc plasmids two distinct fingerprints were obtained suggesting the occurrence of a rearrangement involving one of the *Hind* III recognition sequences in Tn7. Again a change in the plasmid pool appeared to result, with the pattern containing the intact Tn7 persisting while the rearranged plasmid largely disappeared. The altered Tn7 still conferred trimethoprim resistance, but the rearranged plasmid appears to have been at a selective disadvantage compared with the original.

As relatively small numbers of plasmids were examined from what is probably a very large plasmid pool these results must be interpreted with caution; however, it would appear that changes have occurred in the plasmid pool over the period during which the organisms were collected.

Overall, restriction enzyme fingerprinting has extended the groupings based on mol. wt and resistance pattern and has further demonstrated the value of a molecular approach to epidemiology with particular regard to the spread and persistence of trimethoprim plasmids in *E. coli* populations.

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REFERENCES

BEUL, H. A., EICHEL-STREIBER, CH. VON, SCHREINER, M., SCHWINDLING, F. P., WEINBLUM, D., ZOLLNER, E. J. & DIERICH, M. (1985). Characterisation of cryptic plasmids in clinical isolates of *Bacteroides fragilis*. *Journal of Medical Microbiology* **20**, 39–48.

BIRNBOIM, H. C. & DOLY, J. (1979). A rapid alkaline extraction precedure for screening recombinant DNA. *Nucleic Acids Research* **7**, 1513–1523.

DATTA, N. (1977). R-factors in enterobacteriaceae. In *R-factor, Drug Resistance Plasmid* (ed. S. Mitsuhashi) pp. 255–272. Tokyo: Tokyo University Press.

FLEMING, M. P., DATTA, N. & GRUNEBERG, R. N. (1972). Trimethoprim resistance determined by R-factors. *British Medical Journal* **1**, 726–728.

ISH-HOROWITZ, D. & BURKE, J. F. (1981). Rapid and efficient cosmid cloning. *Nucleic Acids Research* **9**, 2989–2998.

KRAFT, C. A., PLATT, D. J. & TIMBURY, M. C. (1983). Distribution and transferability of plasmids in trimethoprim resistant urinary *Escherichia coli*. *Journal of Medical Microbiology* **16**, 433–441.

KRAFT, C. A., PLATT, D. J. & TIMBURY, M. C. (1984). Distribution and transferability of plasmids in trimethoprim-resistant urinary strains of *Escherichia coli*: a comparative study of hospital isolates. *Journal of Medical Microbiology* **18**, 95–105.

KRAFT, C. A., PLATT, D. J. & TIMBURY, M. C. (1985). Trimethoprim resistance in urinary coliforms from patients in the community: plasmids and R-transfer. *Journal of Antimicrobial Chemotherapy* **15**, 311–317.

KRAFT, C. A., TIMBURY, M. C. & PLATT, D. J. (1986). Distribution and genetic location of Tn7 in trimethoprim resistant *E. Coli*. *Journal of Medical Microbiology* **22**, 125–131.

LYON, B. R., IUORIO, J. L., MAY, J. W. & SKURRAY, R. A. (1984). Molecular epidemiology of multi-resistant *Staphylococcus aureus* in Australian hospitals. *Journal of Medical Microbiology* **17**, 79–89.

O'BRIEN, T. F., HOPKINS, J. D., GILLEECE, E. S., MADEIROS, A. A., KENT, R. K., BLACKBURN, B. O., HOLMES, M. B., REARDON, J. P., VERGERONT, J. M., SCHELL, W. L., CHIRSTENSON, E., BISSERT, M. L. & MORSE, E. V. (1982). Molecular epidemiology of antibiotic resistance in *Salmonella* from animals and human beings in the United States. *New England Journal of Medicine* **307**, 1–6.

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

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.

RICHARDS, H. & NUGENT, M. (1979). The incidence and spread of Tn7. In *Plasmids of Medical, Environmental and Commercial Importance* (ed. K. N. Timmis & A. Puhler.) Elsevier/North Holland Biomedical Press.

RUBENS, C. E., FARRAR, W. E. JR., MCGEE, X. A. & SCHAFFNER, W. (1981). Evolution of a plasmid mediating resistance to multiple antimicrobial agents during a prolonged epidemic of nosocomial infections. *The Journal of Infectious Diseases* **143**, 170–181.