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Antimicrobial resistance and the ecology of *Escherichia coli* plasmids

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SUMMARY

Four hundred and seven clinical isolates of *Escherichia coli* were examined for the presence of plasmids. These isolates comprised 189 which were collected irrespective of antimicrobial resistance (VP) and 218 which were collected on the basis of high-level trimethoprim resistance (TPR). The VP isolates were divided into drug sensitive (VPS) and drug-resistant (VPR) subpopulations.

Plasmids were detected in 88% of VP isolates (81% of VPS and 94% of VPR) and 98% of TPR isolates. The distribution of plasmids in both groups and subpopulations was very similar. However, there were small but statistically significant differences between the plasmid distributions. These showed that more isolates in the resistant groups harboured plasmids than in the sensitive subpopulation (VPS) and that the number of plasmids carried by resistant isolates was greater. Multiple drug resistance was significantly more common among TPR isolates than the VPR subpopulation and this was paralleled by increased numbers of plasmids.

Fifty-eight per cent of VPR and 57% of TPR isolates transferred antimicrobial resistance and plasmids to *E. coli* K12. Of the R^+ isolates, 60% carried small plasmids (MW < 20Md) and 52% of these co-transferred with R-plasmids. These results are discussed.

INTRODUCTION

Since the first reports of transferable resistance to antimicrobials in Japan (Watanabe, 1963), the importance of plasmids both to their bacterial hosts and indirectly to man has been progressively appreciated (see Harwood, 1980). Our understanding of the involvement of plasmids in infection has expanded beyond their role in drug resistance with the realization that more and more clinically important characteristics are or can be plasmid-mediated. These include a variety of virulence attributes – colonization ability (Gaastra & de Graaf, 1982), invasiveness (Harris *et al.* 1982), resistance to host defences (Moll, Manning & Timmis, 1980) and toxin production (Wilshaw *et al.* 1980, 1982). Furthermore, plasmid modification of biochemical characters used in the identification of pathogens can result in failure to recognize and treat infections (Smith & Parsell, 1975).

The development of simple and rapid techniques for the physical detection of plasmids which extend to a wide range of bacterial genera has been a major recent advance in this field (Eckhart, 1978; Birnboim & Doly, 1979; Platt & Sommerville,

1981*a*). Apart from the advantage these techniques confer for the correlation of the presence of particular plasmids with bacterial characteristics such as invasiveness (Sansonetti, Kopecko & Formal, 1982), their simplicity also allows the investigation of large numbers of organisms on a prospective basis to study aspects of their ecology. Because of its clinical relevance (see Stuart-Harris & Harris, 1982) and the importance of resistance markers to recombinant DNA technology, plasmid-mediated drug resistance has received most attention. Many studies have demonstrated the clinical importance of plasmids and transposition (Datta, Nugent & Richards, 1980; Platt, Sommerville & Gribben, 1984) but the quantitative aspects remain ill-defined. Early studies relied exclusively on the transfer of resistance markers which, without physical characterization of the plasmids, can lead to misinterpretation of results (Kraft, Platt & Timbury, 1983, 1984).

Transferable resistance in the enterobacteria often accounts for as little as 20% of the resistance detected leaving the greater part unexplained. However, published estimates show that the incidence of transferable resistance varies and depends upon many factors such as the range of genera and the choice of resistance marker studied, and also whether the collection of organisms investigated includes epidemiologically related isolates. Datta *et al.* (1980), in a collection of enterobacteria selected for trimethoprim and gentamicin resistance found 23% and 78% respectively to be transferable. In a survey of *Serratia* spp. isolated in one year, Platt & Sommerville (1981*b*) demonstrated 100% transferability of gentamicin resistance compared to only 50% in the case of carbenicillin resistance, the remaining 50% being due to mutation (Platt, Sommerville & McGroarty, 1983). Furthermore, the choice of recipient strains in conjugation experiments can markedly influence transferability (Sanderson, Janzer & Head, 1981). Although *Escherichia coli* K12 is almost invariably used it may give a falsely low estimate of R-transfer when different enteric genera are used as plasmid donors (Cooksey, Thorne & Farrar, 1976; Platt & Sommerville, 1981*a*).

Previous studies in this department (Kraft, Platt & Timbury, 1983; Sommerville & Platt, unpublished) suggested that R-plasmids represented only a small proportion of the extrachromosomal DNA pool in enterobacteria. However, such a pool could be expected to contribute significantly to the fluidity of resistance genes (Sherratt, 1982). We were therefore prompted to investigate plasmids in isolates of *E. coli* collected irrespective of antimicrobial resistance with the aim of studying the contribution of R-plasmids relative to the overall extrachromosomal gene pool. Here we report the distribution, transfer and mobilization of plasmids in these isolates compared with those obtained from *E. coli* isolates collected on the basis of trimethoprim resistance.

MATERIALS AND METHODS

Bacteria

A total of 407 clinical isolates of *E. coli* were collected between 1979 and 1983, obtained from the routine diagnostic laboratories of Glasgow Royal Infirmary and other hospitals in the Eastern District of Glasgow; of these, 189 were collected from patients with vascular disease (147 from rectal swabs and 42 from wound swabs). The isolates from vascular patients (VP) were divided into 'antibiotic sensitive'

(VPS) and 'antibiotic resistant' (VPR) subpopulations by comparison with *E. coli* K12 in a disk-diffusion sensitivity test as previously described (Platt & Sommerville, 1981*b*). The remaining 218 isolates were *E. coli* from urinary-tract infections collected on the basis of high-level trimethoprim resistance (MIC > 1024 mg/l) (TPR).

E. coli K12 J53-1, J62-1 (Nal^r) and J53-2, J62-2 (Rif^r) (Bachmann, 1972) were used as recipients in conjugation experiments.

Isolation and identification

Standard procedures were used for the isolation of organisms from clinical material (Cruickshank *et al.* 1975). In addition, rectal swabs were plated directly onto CLED agar (Mast DM110) and isosensitest agar (Oxoid CM471) with the following antibiotic disks: carbenicillin 100 μg, cefazolin 30 μg, tetracycline 10 μg, kanamycin 30 μg, streptomycin 10 μg, chloramphenicol 30 μg, sulphamethoxazole 25 μg and trimethoprim 1·25 μg. This facilitated the isolation of resistant strains present in the specimen in small numbers. Any coliforms growing within the inhibition zones were purified before identification by the API 20E system.

Detection and characterization of plasmids

Plasmid DNA was obtained in crude SDS lysates from cultures of both donors and transconjugants grown on nutrient agar (Oxoid CM3). It was subjected to electrophoresis in 0·7 % agarose gels and plasmid molecular weight was estimated as previously described (Platt & Sommerville, 1981*a*).

Resistance transfer to *E. coli* K12 was carried out using a standard broth mating technique and the antimicrobial susceptibility of transconjugants determined by disk sensitivity testing.

Statistical analysis

The chi-squared test was used to compare the plasmid distributions and also the degree of multiple drug resistance. Other results were compared using a chi-squared 2×2 contingency test incorporating Yates correction for continuity (Siegel, 1956).

RESULTS

Four hundred and seven isolates of *E. coli* were studied. Of the 189 VP isolates from vascular patients, 90 were fully sensitive (VPS) to all of the antimicrobial agents tested (ampicillin, tetracycline, chloramphenicol, trimethoprim, sulphamethoxazole, streptomycin, and kanamycin) and 99 were resistant (VPR) to at least one drug. There was no evidence of cross-infection during the collection of these isolates. The 218 isolates collected on the basis of high-level trimethoprim resistance (TPR) were all resistant to at least one other agent. The numbers of isolates with different degrees of multiple resistance are shown in Table 1. There was significantly more multiple drug resistance among the TPR isolates than the VPR subpopulation ($P < 0{\cdot}001$), which shows that the collection of trimethoprim-resistant organisms selects for multiple drug resistance. This is further supported by the observation that the small number of trimethoprim-resistant strains among the VPR isolates were also multiply-resistant.

Table 1. *The distribution of multiple antibiotic resistance among TPR isolates, VPR isolates and the trimethoprim resistant subgroup of VPR isolates*

Number of antimicrobial agents to which isolates were resistant	TPR: isolates collected on the basis of trimethoprim resistance		VPR: resistant subpopulation of vascular patient isolates		Trimethoprim-resistant subgroup of VPR isolates
	Number	(%)	Number	(%)	Number
1	0		18	(18)	0
2	8	(4)	24	(24)	0
3	17	(8)	27	(27)	1
4	52	(24)	12	(12)	1
5	77	(35)	12	(12)	8
6	39	(18)	1	(1)	0
7	25	(11)	5	(5)	5
Total	218	(100)	99	(100)	15

Plasmids were detected in most of the 407 isolates; 98% of TPR isolates, 94% of VPR isolates and 81% of VPS isolates. Some isolates harboured up to seven plasmids. The distribution of plasmids in TPR and VP isolates and VPR and VPS subpopulations is shown in Fig. 1 (*a–d*) respectively. The principle feature of these distributions is their similarity which shows that the acquisition of antimicrobial resistance by *E. coli* has not dramatically affected the number of plasmids harboured. However, there were small but significant differences when the individual distributions were compared. The VPS subpopulation (*d*) contained more plasmid-free isolates than the VPR subpopulation (*c*) ($P < 0{\cdot}01$) and the VPR isolates contained more plasmid-free organisms than the TPR collection (*a*) ($P < 0{\cdot}001$). Comparison of the overall plasmid distributions showed that a significantly greater proportion of VPR isolates contained more plasmids than did the VPS isolates ($P < 0{\cdot}05$) and similarly TPR isolates carried more plasmids than VPR isolates ($P < 0{\cdot}01$).

To exclude the possibility that small plasmids (<20 Md) contributed disproportionately to these data, the distribution of potentially self-transmissible plasmids (>20 Md) (Broda, 1979) was calculated (Fig. 2*a–d*). A comparable similarity in the distributions is apparent which indicates that small plasmids are equally distributed among the different populations. Statistical comparison of the distributions also produced similar results which indicates that the resistance-associated differences are the result of changes in the numbers of large, potentially self-transmissible plasmids.

Resistance transfer was demonstrated from 57% and 58% of TPR and VPR isolates respectively but from only 31% of the VP isolates overall. Comparison of the plasmid distribution in TPR and VPR isolates that transferred resistance to *E. coli* K12, showed no significant difference in the numbers of plasmids present. However, there were considerably more large plasmids (>20 Md molecular weight) in the TPR isolates than in VPR subpopulation; this difference was significant ($P < 0{\cdot}01$). The mobilization of small plasmids from TPR and VPR isolates that transferred resistance is summarized in Table 2. There were no significant differences between the two collections as regards the number of small plasmids

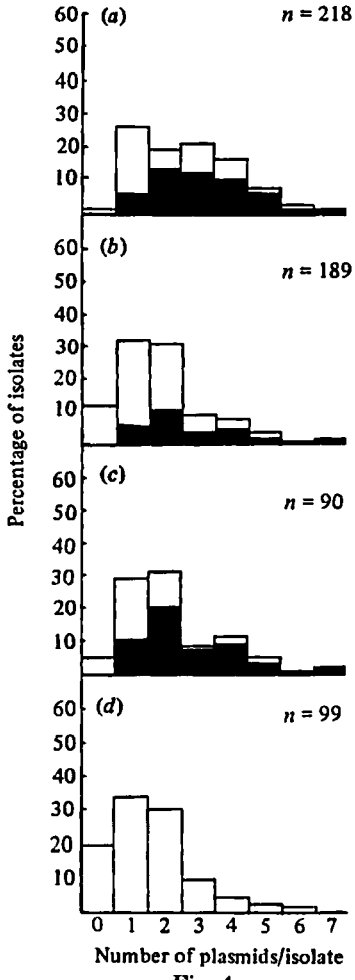

Fig. 1.

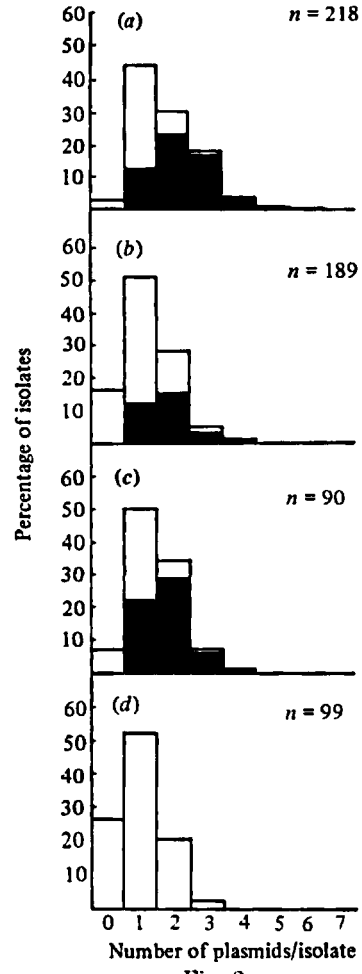

Fig. 2.

Fig. 1. The distribution of plasmids in *E. coli* collected on the basis of high-level trimethoprim resistance (TPR) (*a*) and isolates collected from patients with vascular disease (VP) (*b*). (*c*) and (*d*) are resistant and sensitive subpopulations of VP isolates respectively. ■, Proportion of isolates harbouring self-transmissible R-plasmids.

Fig. 2. The distribution of plasmids > 20 Md in *E. coli* collected on the basis of high level trimethoprim resistance (TPR) (*a*) and isolates collected from patients with vascular disease (VP) (*b*). (*c*) and (*d*) are resistant and sensitive subpopulations of VP isolates respectively. ■, Proportion of isolates harbouring self-transmissible R-plasmids.

present or the number mobilized ($P > 0{\cdot}05$). Over half the isolates contained small plasmids and 52% of these were mobilized by the R-plasmid(s) present.

DISCUSSION

Our most notable findings were the large number of plasmids in the fully sensitive isolates and the broad similarity of the distribution of plasmids in sensitive and resistant isolates. This suggests that R-plasmids comprise only a

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Table 2. *Mobilization of small plasmids (< 20 Md) by transferable R-plasmids from VPR isolates and isolates collected on the basis of trimethoprim resistance (TPR)*

	VPR		TPR	
	Isolates	Plasmids	Isolates	Plasmids
No. of isolates transferring resistance	58	—	124	—
Small plasmids present	33 (57%)	62	79 (64%)	124
Small plasmids mobilized	17 (29%) (52%)*	32 (52%)	41 (33%) (52%)*	69 (56%)
Small plasmids not mobilized	16 (28%)	30 (48%)	38 (31%)	55 (44%)

* Small plasmids mobilized as a proportion of small plasmids detected.

Table 3. *The increased plasmid content of resistant populations (VPR and TPR) of* E. coli *relative to the sensitive population (VPS)*

	E. coli population		
	VPS	VPR	TPR
Total no. of plasmids detected	141	226	590
No. of isolates	90	99	218
No. of isolates harbouring plasmids	73	93	214
No. of plasmids per isolate calculated after exclusion of plasmid free isolates from each population	1·93	2·43	2·76
Plasmids per isolate as a percentage of VPS	100	126	143

minor part of the extrachromosomal gene pool of *E. coli*. Our results also indicate that the acquisition of resistance has resulted in a small but significant change in the distribution of plasmids.

Plasmids that were too small to be self-transmissible, appear to play a minimal role in antibiotic resistance. However, they were widely distributed among our isolates and the frequency with which they were mobilized was comparable to the transfer frequency of R-plasmids (Table 2). This suggests that the lack of transfer genes does not materially diminish their mobility and that their contribution to evolution within the accessory gene pool of bacteria may have been underestimated.

Early work with collections of resistant enterobacteria led to the belief that the use of antibiotics results in the formation and dissemination of conjugative R-plasmids (Anderson, 1965). Our results support this suggestion. In particular, when each of the resistant groups was compared with the sensitive VPS group there was a 26% increase in the total number of plasmids carried by the VPR subpopulation whereas the corresponding increase in the TPR collection was 43% (Table 3). Thus, it appears that the acquisition of drug resistance was associated with an increase in the number of plasmids harboured; an increase in the incidence of multiple drug resistance between the two resistant collections was paralleled by a further increase in plasmid numbers. It is interesting that despite these differences the proportion of transferable resistance remained unchanged.

Hughes & Datta (1983) and Datta & Hughes (1983) demonstrated that many bacteria isolated in the pre-antibiotic era contain conjugative plasmids. They

concluded that conjugative plasmids were as common then as they are in drug-sensitive strains today and suggested that the R-factors prevalent today are derived from them. Furthermore, their interpretation of these results was in cotrast to Anderson's conclusions (1965). However, our finding of large numbers of plasmids in sensitive isolates together with the increased number of plasmids in resistant isolates reconciles both views. Thus, a small number of transfer events, which occur under the selection pressure imposed by antibiotic usage, can provide a large potential for macro- and micro-evolution of plasmids by the mechanisms described by Cohen *et al.* (1977).

REFERENCES

Anderson, E. S. (1965). Origin of transferable drug-resistance factors in the Enterobacteriaceae. *British Medical Journal* ii, 1289–1291.

Bachmann, B. J. (1972). Pedigrees of some mutant strains of *Escherichia coli* K-12. *Bacteriological Reviews* **36**, 525–557.

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

Broda, P. (1979). *Plasmids*, p. 14. Oxford: W. H. Freeman.

Cohen, S. N., Brevet, J., Cabello, F., Chang, A. C. Y., Chou, J., Kopecko, D. J., Kretschmer, P. J., Nisen, P. & Timmis, K. N. (1978). Macro- and microevolution of bacterial plasmids. In *Microbiology* (ed. D. Schlessinger), pp. 217–220. Washington: A.S.M.

Cooksey, R. C., Thorne, G. M. & Farrar, W. E. Jr. (1976). R-factor mediated antibiotic resistance in *Serratia marcescens*. *Antimicrobial Agents and Chemotherapy* **10**, 123–127.

Cruickshank, R., Duguid, J. P., Marmion, B. P. & Swain, R. H. A. (1975). *Medical Microbiology*. Volume 2. *The Practice of Medical Microbiology*, 12th ed. Edinburgh: Churchill Livingstone.

Datta, N. & Hughes, V. M. (1983). Plasmids of the same Inc groups in Enterobacteria before and after the medical use of antibiotics. *Nature* **306**, 616–617.

Datta, N., Nugent, M. & Richards, H. (1980). Transposons encoding trimethoprim or gentamicin resistance in medically important bacteria. *Cold Spring Harbour Symposium on Quantitative Biology* **45**, 45–51.

Eckhardt, T. (1978). A rapid method for the identification of plasmid DNA in bacteria. *Plasmid* **1**, 584–588.

Gaastra, W. & de Graaf, F. K. (1982). Host-specific fimbrial adhesins of noninvasive enterotoxigenic *Escherichia coli* strains. *Microbiological Reviews* **46**, 129–161.

Harris, J. R., Wachsmuth, I. K., Davis, B. R. & Cohen, M. L. (1982). High molecular weight plasmid correlates with *Escherichia coli* enteroinvasiveness. *Infection and Immunity* **37**, 1295–1298.

Harwood, C. R. (1980). Plasmids. In Society for Applied Bacteriology Symposium No. 8: *Microbial Classification and Identification* (ed. M. Goodfellow and R. G. Board), pp. 27–53. London: Academic Press.

Hughes, V. M. & Datta, N. (1983). Conjugative plasmids in bacteria of the 'pre-antibiotic' era. *Nature* **302**, 725–726.

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*. (In the Press.)

Moll, A., Manning, P. A. & Timmis, K. N. (1980). Plasmid determined resistance to serum bactericidal activity: a major outer membrane protein, the *tra* T gene product, is responsible for plasmid specified serum resistance in *Escherichia coli*. *Infection and Immunity* **28**, 359–367.

Platt, D. J., Sommerville, J. S. & McGroarty, J. A. (1983). Characterisation of spontaneous resistant variants of *Serratia marcescens* selected in the presence of carbenicillin. *Journal of Antimicrobial Chemotherapy* **12**, 329–335.

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

PLATT, D. J. & SOMMERVILLE, J. S. (1981*b*). *Serratia* species isolated from patients in a general hospital. *Journal of Hospital Infection* **2**, 341–348.

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

SANDERSON, L. E., JANZER, J. & HEAD, J. (1981). Influence of lipopolysaccharide and protein in the cell envelope on recipient capacity in conjugation of *Salmonella typhimurium*. *Journal of Bacteriology* **148**, 283–293.

SANSONETTI, P. J., KOPECKO, D. J. & FORMAL, S. B. (1982). Involvement of a plasmid in the invasive ability of *Shigella flexneri*. *Infection and Immunity* **35**, 852–860.

SHERRATT, D. J. (1982). The maintenance and propagation of plasmid genes in bacterial populations. *Journal of General Microbiology* **128**, 655–661.

SIEGEL, S. (1956). *Non-parametric Statistics for the Behavioural Sciences*. London: McGraw-Hill.

SMITH, H. WILLIAMS & PARSELL, Z. (1975). Transmissible substrate utilizing ability in the enterobacteria. *Journal of General Microbiology* **87**, 129–140.

STUART-HARRIS, C. H. & HARRIS, D. M. (1982). The control of antibiotic-resistant bacteria. London: Academic Press.

WATANABE, T. (1963). Infectious heredity of multiple drug resistance in bacteria. *Bacteriological Reviews* **27**, 87–115.

WILSHAW, G. A., BARCLAY, E. A., SMITH, H. R., MCCONNELL, M. M. & ROWE, B. (1980). Molecular comparison of plasmids encoding heat-labile enterotoxin isolated from *Escherichia coli* strains of human origin. *Journal of Bacteriology* **143**, 168–175.

WILSHAW, G. A., SMITH, H. R., MCCONNELL, M. M., BARCLAY, E. A., KRNJULAC, J. & ROWE, B. (1982). Genetic and molecular studies of plasmids coding for colonization factor antigen I and heat-stable enterotoxin in several *Escherichia coli* serotypes. *Infection and Immunity* **37**, 858–868.