Distribution and genetic location of Tn7 in trimethoprim-resistant *Escherichia coli*

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Summary. A series of 178 strains of *Escherichia coli*, highly resistant to trimethoprim, isolated from hospital patients and patients in the community between 1979 and 1983, was examined for the presence of Tn7 on a plasmid or on the chromosome only, by transposition to RP4 and restriction endonuclease digestion with *Hind* III. Of the isolates, 57% carried Tn7. Comparison of hospital isolates from 1979 to 1980 and 1982 showed that although the proportions that carried Tn7 were similar (63% and 57%) there had been a significant change in the genetic location of the transposon. The proportion of plasmid-mediated Tn7 had fallen from 62% to 30% with a corresponding rise in Tn7 located exclusively on the chromosome from 38% to 70%. This change may be the result of continuing transposition of Tn7 from plasmids to the bacterial chromosome followed by plasmid loss. The consequent reduction in the mobility of trimethoprim-resistance genes may in turn lead to changes in the incidence of resistance.

Introduction

Transposon Tn7, which mediates high level resistance to trimethoprim and low level resistance to streptomycin, was first detected in Britain in IncI plasmids (Barth *et al.*, 1976; Barth and Datta, 1977). It has since been reported in plasmids of several other incompatibility groups including IncM, IncC and IncX (Datta *et al.*, 1981). Tn7 can transpose readily between replicons, including the *Escherichia coli* chromosome which has a 'hot spot', and with the continued use of trimethoprim, worldwide dissemination of the transposon has been predicted (Richards and Nugent, 1979). The transposon has recently been demonstrated in isolates from Finland and Sweden (Dornbusch and Hagelberg, 1983).

Recently, an increasingly large proportion of trimethoprim high level resistance in coliforms has been found to be non-transferable (Towner *et al.*, 1980; Kraft *et al.*, 1984). Towner *et al.* (1982) demonstrated transposition of trimethoprim resistance in *E. coli* isolates that lacked self-transmissible R-plasmids. These findings suggest that resistance may be carried on non-transferable (tra⁻) plasmids or be mediated by a transposable element inserted into the bacterial chromosome.

In the present study we have investigated the incidence of Tn7 in highly trimethoprim resistant *E.*

coli strains isolated in the course of earlier studies from hospital patients and from patients attending general practitioners (Kraft *et al.*, 1983, 1984, 1985). The aim was to determine the proportion of high level resistance to trimethoprim coded for by Tn7 in a large series of isolates and to determine directly how many isolates carried the transposon exclusively in a chromosomal location and how many on a plasmid. We also investigated the possibility that a change had occurred in the relative proportions of isolates that carried Tn7 in these two locations during the period 1979–1982.

Materials and methods

Design of the study

Clinical isolates of *E. coli* highly resistant to trimethoprim (MIC > 1024 µg/ml) were collected during three earlier studies (see table I) and were divided into two groups: those which transferred trimethoprim resistance to *E. coli* K12 (TT) and those which failed to transfer resistance to the drug in a standard 24-h broth mating at 37°C (TNT), irrespective of their ability to transfer other resistance determinants (for details of transfer method see Kraft *et al.*, 1983). There were 89 isolates in the TT group and 97 in the TNT group. Standard strains and plasmids are listed in table II.

Media

Cystine-Lactose-Electrolyte-Deficient (CLED) Agar (Mast) was used for growth and maintenance of orga-

Table I. Clinical isolates of *E. coli* included in the study

Source of isolates	Number of isolates highly resistant to trimethoprim	Number of isolates studied with		Total	Reference
		transferable trimethoprim resistance (TT)	non-transferable trimethoprim resistance (TNT)		
Hospital 1979-80	88	43*	26	69	Kraft <i>et al.</i> , 1983
Hospital 1982	98	35†	54	89	Kraft <i>et al.</i> , 1984
General practice 1981-83	32	11	17	28	Kraft <i>et al.</i> , 1985
Total	218	89	97	186	

* 44 isolates tested—one transferred trimethoprim resistance to *E. coli* K12 but no plasmid DNA could be demonstrated in the transconjugant which was therefore excluded from the present study.

† 34 isolates tested—one transferred two trimethoprim resistance plasmids which differed in mol. wt and in resistance pattern.

Table II. Standard strains and plasmids

Strain	Plasmid	Antibiotic resistance†	Source or reference
<i>E. coli</i> K12 J62-2	None	Rif	Bachmann, 1972
<i>E. coli</i> K12 J53-2	None	Rif	Bachmann, 1972
<i>E. coli</i> K12 J62-1	None	Nal	Bachmann, 1972
<i>E. coli</i> K12 J53-1	None	Nal	Bachmann, 1972
<i>E. coli</i> K12 J62	RP4	Tc Cb Km	N. Datta*
<i>E. coli</i> K12 J53	RP4	Tc Cb Km	N. Datta*
<i>E. coli</i> K12 J62::Tn7	None	Tp Sm	H. Richards*
<i>E. coli</i> K12 J62	RP4::Tn7	Tp Sm Tc Cb Km	This laboratory
<i>P. aeruginosa</i> NCTC 10662	None		

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† Rif—rifampicin, Nal—nalidixic acid, Tc—tetracycline, Cb—carbenicillin, Km—kanamycin, Tp—trimethoprim, Sm—streptomycin.

nisms and Isosensitest Agar and Broth (Oxoid) for sensitivity tests. Nutrient Agar (NA; Oxoid), blood agar (Oxoid Columbia Agar with 5% defibrinated horse blood), Brain Heart Infusion Broth (BHI; Oxoid) and Nutrient Broth (CM1; Oxoid) were used for growth of organisms for DNA extraction. BHI was used for broth conjugation and CLED agar for surface mating.

The following antimicrobial agents ($\mu\text{g/ml}$) were incorporated into CLED agar for the selection of transconjugants: rifampicin (Rif) 50 (with ascorbic acid 5 $\mu\text{g/ml}$); trimethoprim (Tp) 30 (for transfer into *E. coli*) or 1024 (for transfer into *Pseudomonas aeruginosa*) (with uridine 10 and 100 $\mu\text{g/ml}$ respectively); tetracycline (Tc) 10; kanamycin (Km) 25 (*E. coli*) or 120 (*P. aeruginosa*); carbenicillin (Cb) 400; nalidixic acid (Nal) 10; cefrimide (Cet) 300.

Antimicrobial sensitivity tests and test for colicin production

Antimicrobial sensitivity testing was performed as

described previously (Kraft *et al.*, 1983). To distinguish between intrinsic and high level resistance to trimethoprim in *P. aeruginosa*, 50 μl of trimethoprim (10-24 mg/ml) was added to a well in the sensitivity plate.

The production by clinical isolates of *E. coli* of colicins active against *E. coli* K12 was tested by the agar overlay method (Barth *et al.*, 1978). The isolates were stabbed into NA plates and overlaid with 10 ml of 0.5% soft agar containing 10 μl of an overnight broth culture of *E. coli* K12. Clear zones in the indicator lawn after overnight incubation at 37°C were taken to indicate colicin-like activity.

Translocation of trimethoprim resistance into RP4

RP4 was transferred into the clinical isolates in a standard 24-h broth mating at 37°C, with trimethoprim and tetracycline, kanamycin or carbenicillin selection. The plasmid was then re-transferred to an appropriate strain of *E. coli* K12 with selection for trimethoprim resistance or for tetracycline resistance (control). When

indigenous plasmids co-transferred with putative RP4::Tn7 the latter was separated by transfer into *P. aeruginosa* and selection with trimethoprim and ceftriadime, and then re-transfer to *E. coli* K12 and selection with trimethoprim and rifampicin. A surface mating method was used (Bradley *et al.*, 1980).

Detection of plasmid DNA

Plasmid DNA was extracted, separated by gel electrophoresis and the mol. wt determined as described previously (Platt and Sommerville, 1981).

Restriction enzyme analysis

Organisms were grown in BHI or CM1. Plasmid DNA was extracted by the alkaline-SDS method of Birnboim and Doly (1979) as modified by Ish-Horowitz and Burke (1981). Furthermore the preparations were treated with 7.5 M ammonium acetate before ethanol precipitation. The plasmid DNA was digested with *Hind* III (BRL) in accordance with the manufacturer's instructions. The DNA fragments were separated by electrophoresis on horizontal 0.7% agarose gels in tris-borate buffer, pH 8.0, incorporating ethidium bromide 0.3 µg/ml. Gels were run at 18 mA for 16–18 h. Phage Lambda DNA (BRL) was included to provide mol. wt standards and RP4::Tn7 was run as a control.

TT group

E. coli K12 transconjugants into which trimethoprim-resistance plasmids from the TT group had been transferred were tested for the presence of Tn7 by restriction endonuclease digestion with *Hind* III. When a transconjugant plasmid did not correspond in mol. wt to any plasmid in the original clinical isolate, plasmid DNA from the original isolate was also tested for the presence of a plasmid-coded Tn7.

TNT group

Plasmid DNA from all isolates in the TNT group was screened for the presence of Tn7 by digestion with *Hind* III to determine whether Tn7 was carried on *tra*⁻ plasmids. The isolates were then screened for the presence of Tn7 in the bacterial chromosome by testing for translocation of trimethoprim resistance to RP4. Thirty two isolates resistant to tetracycline, kanamycin and carbenicillin were excluded from the TNT group, because it was not possible to select for incoming RP4.

When trimethoprim resistance translocated to RP4, the resulting plasmids were tested for Tn7 by restriction endonuclease digestion with *Hind* III. This was to distinguish between isolates that lacked a plasmid-coded Tn7 and in which a Tn7 translocating to RP4 must have come from the bacterial chromosome, and isolates that carried a plasmid-coded Tn7, in which transposition to RP4 together with lack of transfer in earlier experiments suggested location of the transposon on a *tra*⁻ plasmid.

Recognition of Tn7

For the purposes of the present study the following criteria have been taken as evidence of the presence of Tn7 or a similar transposon (after Datta and Richards, 1981): (i) Resistance to trimethoprim (MIC ≥ 1024 µg/ml) and to streptomycin; (ii) in TNT isolates, translocation of trimethoprim- and streptomycin-resistance determinants to RP4 or to an indigenous plasmid, accompanied by an increase in mol. wt of *c.* 10×10^6 ; (iii) after digestion of plasmid DNA with *Hind* III, the appearance of two characteristic fragments (mol. wt 1.4×10^6 and 1.7×10^6 respectively) which corresponded on gel electrophoresis to fragments from control RP4::Tn7.

Statistical analysis

A chi-squared 2×2 contingency test was applied, incorporating Yates' correction for continuity (Siegel, 1956).

Results

Plasmid-mediated Tn7

Analysis of 89 transferable (*tra*⁺) trimethoprim-resistance plasmids in *E. coli* K12 transconjugants showed that 46 carried Tn7. The plasmids analysed included 80, each of which corresponded in mol. wt to a plasmid in its respective clinical donor isolate (fig. 1); 37 of these carried Tn7. The remaining nine plasmids did not correspond in mol. wt to any plasmid in the donor isolate (fig. 2). The clinical isolates from which these were obtained were also examined for plasmid-mediated Tn7 to determine the original location of the transposon. Tn7 was present in all of these nine plasmids. However, in six of the nine isolates from which these plasmids had been transferred, *Hind* III digests of plasmid DNA failed to show the fragments characteristic of Tn7, indicating that the transposon was located on the

Fig. 1. Agarose (0.7%) gel electrophoresis of plasmid DNA in crude lysates: A and B—two *E. coli* K12 transconjugants; C—clinical *E. coli* isolate (GRI50582).

Fig. 2. Agarose gel (0.7%) showing: A—plasmid DNA extracted from a clinical isolate of *E. coli* (GRI 183); B and C—two *E. coli* K12 transconjugants. The mol. wt of transconjugant plasmids does not correspond to any plasmid in the donor.

bacterial chromosome. These nine isolates included four of the five isolates described previously (Kraft *et al.*, 1984), in which the transconjugant plasmid had a mol. wt *c.* 10×10^6 larger than one of those in the clinical isolate and transposition of Tn7 from the chromosome was suspected. One transconjugant plasmid from strain GRI 1482 had a mol. wt 10×10^6 greater than the larger of the two plasmids present in the clinical isolate. This larger plasmid was transferable and carried ampicillin resistance (Kraft *et al.*, 1984). The clinical isolate was found to carry plasmid-mediated Tn7, which indicated transposition from the smaller *tra*⁻ plasmid to the larger *tra*⁺ plasmid.

Forty of 89 (45%) *tra*⁺ trimethoprim R-plasmids in the clinical isolates carried Tn7. A larger proportion of plasmids from the first (1979–80) hospital collection carried Tn7 than did those from the second (1982) collection: 25 of 43 (58%) and 13 of 35 (37%) respectively. This difference was not statistically significant ($p > 0.1$). Two of 11 plasmids from the community isolates carried Tn7 (18%).

Isolates with non-transferable trimethoprim resistance

RP4 was transferred successfully into 93 of 97 TNT isolates. From 82 of these 93 isolates RP4 was re-transferred to *E. coli* K12. By use of trimethoprim selection, 49 of these transconjugants were found to harbour RP4 that carried translocated trimethoprim and streptomycin resistance. These plasmids had a mol. wt of *c.* 46×10^6 and in all but two cases, digestion with *Hind* III showed the two restriction fragments characteristic of Tn7 (fig. 3).

Fig. 3. Agarose (0.7%) gel electrophoresis of *Hind* III restriction digests of purified plasmid DNA: A—phage lambda DNA; B—RP4; C—RP4::Tn7; D–G—four *E. coli* K12 transconjugants containing RP4 with transposed trimethoprim and streptomycin resistance.

Fig. 4. Agarose (0.7%) gel electrophoresis of *Hind* III restriction digests of purified plasmid DNA: A—phage lambda DNA; B—RP4; C—RP4::Tn7; D–J—six *E. coli* K12 transconjugants containing RP4 with transposed trimethoprim and streptomycin resistance. Two of these (*) lack the larger of the two fragments characteristic of Tn7.

In the two remaining cases, the 1.7×10^6 -mol. wt fragment was absent (fig. 4) although no change in the mol. wt of the plasmid was detected. However, an additional fragment of mol. wt 3.8×10^6 was seen in the digests of both plasmids. None of the TNT isolates showed the characteristic Tn7 fragments on *Hind* III digestion of their plasmid DNA confirming the chromosomal location of the transposon.

Table III. Trimethoprim-resistance plasmids transferred by clinical isolates of *E. coli* containing RP4

Clinical isolate	Plasmid mean mol. wt (10 ⁶)	Resistance pattern	Self transferability	Presence of Tn7
GRI 10379	62	Tp Su Sm Tc Ap	—	—
GRI 1880	30	Tp Sm	—	—
GRI 3780	36	Tp Su Sm Ap	+	+
GRI 8281	50	Tp Sm Cm Km	+	—
GRI 13781	< 10	Tp Sm (Tc)*	—	—
GRI 3882	62	Tp Su Sm Ap	+	+
GRI 12682	62	Tp Su Sm Ap	+	—
GRI 183	46†	Tp	+	+

Tp—trimethoprim, Su—sulphamethoxazole, Sm—streptomycin, Tc—tetracycline, Ap—ampicillin, Cm—chloramphenicol, Km—kanamycin.

* RP4 present in transconjugant; therefore it was not possible to determine if this plasmid carried Tc resistance.

† Tn7 apparently acquired from chromosome—see text.

In a further eight TNT isolates a donor plasmid that carried trimethoprim resistance was transferred. Five of these plasmids were found subsequently to be conjugative. The remainder appear to have been mobilised co-incidentally by RP4 and none carried Tn7. The characteristics of these eight plasmids are shown in table III. One plasmid gave the *Hind* III fragments characteristic of Tn7 even though it conferred resistance to trimethoprim but not to streptomycin.

Of the 13 isolates that failed either to acquire or to re-transfer RP4, or to transfer an indigenous trimethoprim-resistance plasmid, eight showed colicin-like activity against *E. coli* K12.

Changes in the genetic location of Tn7

Of 218 highly trimethoprim resistant *E. coli* strains that had been collected, 173 (79%) of the clinical isolates or transconjugants derived from them were examined in the present study. (The 13 TNT isolates that failed either to acquire or to re-transfer RP4 or to transfer an indigenous trimethoprim-resistance plasmid were excluded from this total.) The strains examined represented 75% (66 of 88) and 84% (82 of 98) of the isolates from the 1979–80 and 1982 hospital collections respectively and 78% (25 of 32) of isolates from general practice. Since there was no significant difference between the

Table IV. The genetic location of Tn7 in trimethoprim-resistant *E. coli*

Genetic location of Tn7	Number of isolates from			Total
	Hospital 1979–80	1982	General practice 1981–83	
<i>Tn7 located on bacterial chromosome</i>				
Demonstrated by Tn7 translocation to RP4	13	30	6	49
Inferred from Tn7 translocation to clinical plasmid	3	3	1	7
Total (%)	16 (38)	33 (70)	7 (78)	56
<i>Plasmid located Tn7</i>				
Tra ⁺ plasmids	26	13	2	41
Tra [−] plasmids	0	1	0	1
Total (%)	26 (62)	14 (30)	2 (22)	42
<i>Total Tn7 (%)</i>	42 (100)	47 (100)	9 (100)	98

proportion of isolates examined from each collection ($p > 0.2$) the proportions of isolates that carried Tn7 were compared and changes in the genetic location of the transposon analysed.

Of the 173 isolates examined 98 (57%) contained Tn7. The proportions of isolates that harboured Tn7 in the two hospital collections were similar—42 (63%) of the 1979–80 isolates and 47 (57%) in those from 1982. The corresponding figure for isolates from general practice was 9 (36%) but this difference was not statistically significant ($p > 0.05$). The genetic location of Tn7 in isolates from the three collections is shown in table IV. When the two hospital collections were compared, there was a highly significant difference in the proportions of plasmid and chromosomally-mediated Tn7. Between 1979–80 and 1982 the proportion of plasmid-mediated Tn7 had fallen from 62% to 30% with a corresponding rise in chromosomally-located Tn7 from 38% to 70% ($p < 0.005$) (table IV). Seven out of nine isolates from the community carried Tn7 on the chromosome and two on a plasmid. Although they resemble the proportions in the 1982 hospital isolates these numbers are too small to permit statistical comparison.

Discussion

The most notable finding was the significant change between 1979–80 and 1982 in the genetic location of Tn7 from a predominantly plasmid location to a location exclusively on the bacterial chromosome in isolates which did not carry Tn7 on a plasmid. This provides evidence in support of the earlier hypothesis that the increasing incidence of non-transferable, trimethoprim high level resistance is the result of transposition of Tn7 from plasmids to the bacterial chromosome followed by plasmid loss (Towner, 1982; Kraft *et al.*, 1984). Among the isolates described here, Tn7 was found on a single *tra*⁺ plasmid, although we cannot explain why such plasmids are not more common.

However, Tn7 accounted for only 57% of the trimethoprim high level resistance observed. Resistance in the remaining isolates could be mediated by several different mechanisms. Some isolates may carry a defective Tn7 that either fails to transpose to RP4 or that, when plasmid mediated, lacks the *Hind* III restriction sites so that the characteristic fragments are not seen. We conclude that some of our isolates carried modified Tn7 because in two instances translocation occurred to RP4 of the appropriate resistance markers and a segment of DNA of appropriate mol. wt, but one of the characteristic fragments was missing and an addi-

tional larger fragment was seen. This probably arose by the insertion of a 2.1×10^6 mol. wt segment of DNA into the larger (1.7×10^6) Tn7 fragment. Furthermore, the characteristic restriction fragments were obtained from plasmids that lacked streptomycin resistance; these may carry a transposon resembling Tn78 (Datta *et al.*, 1979). Alternatively, some of the plasmid-mediated resistance may be due to Tn402 which specifies trimethoprim resistance only (Shapiro and Sporn, 1977) and which does not transpose readily to other replicons *in vitro* (Datta and Richards, 1981). Other genetic mechanisms may also be involved, because at least three different trimethoprim-resistant dihydrofolate reductases have been described (Fling *et al.*, 1982).

Although we were able to show in some clinical isolates that Tn7 was present in the chromosome but not in any of the plasmids, the converse could not be demonstrated. Thus, in isolates that carried Tn7 on a *tra*⁺ plasmid an additional copy may have been present in the chromosome. This situation might be maintained, without loss of the plasmid, if there was selection for plasmid resistance markers other than trimethoprim. Alternatively, plasmids that carry Tn7 could be maintained if there were genetic changes in the transposon that prevented transposition. Plasmids that carry other trimethoprim-resistance determinants may be retained in the population if these determinants do not transpose readily to the chromosome. Our results show that there was an increase between 1979–80 and 1982 in the proportion of plasmids that carried resistance determinants other than Tn7.

Our results indicate that in addition to transposition of Tn7 from plasmid to chromosome, the reverse also occurs. With some clinical isolates in which plasmid-mediated Tn7 could not be demonstrated, conjugation and trimethoprim selection allowed the isolation of a very small number of transconjugants that did carry plasmid-coded Tn7. The most probable sequence of events is acquisition of a Tn7 carrying plasmid, transposition of Tn7 to the chromosome, loss of the Tn7 carrying plasmid followed by transposition of Tn7 from the chromosome to another plasmid. Thus transposition to the chromosome, which stabilises the resistance (Sherratt, 1982), retains the potential for transfer. Such strains, however, were rare in our collection and probably arose as a result of laboratory manipulation, but the process may have a parallel *in vivo* in conditions of trimethoprim selection.

Fewer isolates from the community carried Tn7 than did hospital isolates. However, in the community isolates, as in the second hospital collection,

exclusively chromosomal Tn7 predominated over plasmid-mediated Tn7, which suggests that the process of transposition to the chromosome and subsequent plasmid loss is occurring in community isolates also.

In conclusion, the present study showed that Tn7 accounted for over half the trimethoprim high level resistance detected. There was a significant change in the genetic location of Tn7 with an increasing

tendency for the transposon to be located exclusively on the chromosome. This may result in a decrease in the mobility of Tn7 and thus to changes in the incidence of resistance.

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REFERENCES

- Bachmann B J 1972 Pedigrees of some mutant strains of *Escherichia coli* K12. *Bacteriological Reviews* **36**:525–557.
- Barth P T, Datta N, Hedges R W, Grinter N J 1976 Transposition of a deoxyribonucleic acid sequence encoding trimethoprim and streptomycin resistances from R483 to other replicons. *Journal of Bacteriology* **125**:800–810.
- Barth P T, Datta N 1977 Two naturally occurring transposons indistinguishable from Tn7. *Journal of General Microbiology* **102**:129–134.
- Barth P T, Richards H, Datta N 1978 Copy numbers of coexisting plasmids in *Escherichia coli* K12. *Journal of Bacteriology* **135**:760–765.
- Birnboim H C, Doly J 1979 A rapid alkaline extraction procedure for screening recombinant plasmid DNA. *Nucleic Acids Research* **7**:1513–1523.
- Bradley D E, Taylor D E, Cohen D R (1980) Specification of surface mating systems among conjugative drug resistance plasmids in *Escherichia coli* K12. *Journal of Bacteriology* **143**:1466–1470.
- Datta N, Hughes V M, Nugent M E, Richards H 1979 Plasmids and transposons and their stability and mutability in bacteria isolated during an outbreak of hospital infection. *Plasmid* **2**:182–196.
- Datta N, Nugent M, Richards H 1981 Transposons encoding trimethoprim or gentamicin resistance in medically important bacteria. Cold Spring Harbor Symposia on Quantitative Biology **XLV**:45–51.
- Datta N, Richards H 1981 Trimethoprim resistant bacteria in hospital and in the community: spread of plasmids and transposons. In Levy S B *et al.* (eds) *Molecular biology, pathogenicity and ecology of bacterial plasmids*. Plenum Press, New York, pp 21–30.
- Dornbusch K, Hagelberg A 1983 Transferable trimethoprim resistance in urinary tract pathogens isolated in Finland and Sweden. *Scandinavian Journal of Infectious Diseases* **15**:285–291.
- Fling M E, Walton L, Elwell L P 1982 Monitoring of plasmid-encoded, trimethoprim-resistant dihydrofolate reductase genes: detection of a new resistant enzyme. *Antimicrobial Agents and Chemotherapy* **22**:882–888.
- Ish-Horowicz 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.
- Platt D J, Sommerville J S 1981 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.
- Richards H, Nugent M 1979 The incidence and spread of transposon 7. In: Timms K W, Puhler A (eds) *Plasmids of medical, environmental and commercial importance*. Elsevier/North Holland Biomedical Press, pp 195–198.
- Shapiro J A, Sporn P 1977 Tn402: a new transposable element determining trimethoprim resistance that inserts in bacteriophage lambda. *Journal of Bacteriology* **129**:1632–1635.
- 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*. McGraw Hill Book Company Inc. New York.
- Towner K J, Pearson N J, Pinn P A, O'Grady F 1980 Increasing importance of plasmid-mediated trimethoprim resistance in enterobacteria: two six month clinical surveys. *British Medical Journal* **280**:517–519.
- Towner K J 1982 Resistance to trimethoprim among urinary tract isolates in the United Kingdom. *Reviews of Infectious Diseases* **4**:456–460.
- Towner K J, Venning B M, Pinn P A 1982 Occurrence of transposable trimethoprim resistance in clinical isolates of *Escherichia coli* devoid of self-transmissible resistance plasmids. *Antimicrobial Agents and Chemotherapy* **21**:336–338.