Occurrence of Transposable Trimethoprim Resistance in Clinical Isolates of *Escherichia coli* Devoid of Self-Transmissible Resistance Plasmids

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Fifty trimethoprim-resistant clinical isolates of *Escherichia coli*, devoid of self-transmissible trimethoprim resistance plasmids, were examined for the presence of trimethoprim resistance transposons. Trimethoprim resistance was mobilized from 12 strains by transposition onto plasmid RP4. The trimethoprim resistance transposons isolated comprised two groups: those with and without linked streptomycin resistance.

Recently, the first report appeared of a clinical isolate of *Escherichia coli* owing its trimethoprim resistance to a chromosomally located Tn7-like transposon in the absence of a detectable resistance plasmid (9). In an attempt to further assess the importance in clinical isolates of this form of trimethoprim resistance, this study reports on the different mechanisms of resistance encountered in 50 clinical isolates of *E. coli* which were resistant to trimethoprim ($\geq 1,024$ mg/liter) but which failed to transfer their resistance in conjugation experiments with *E. coli* K-12.

The 50 clinical isolates of *E. coli* were isolated from infected urine specimens received at this laboratory during 1979 and 1980 from 10 hospitals and 9 general practitioners located within a 20-mile radius of Nottingham. Repeat specimens or obvious examples of cross infection were excluded. Each isolate also had to meet the following criteria: (i) no requirement for thymine (2); (ii) a minimum inhibitory concentration of $\geq 1,024$ mg of trimethoprim per liter, which is normally considered characteristic of a trimethoprim resistance plasmid; (iii) failure to transfer trimethoprim resistance to *E. coli* K-12 in 18-h conjugation experiments (10); and (iv) susceptibility to other appropriate antibiotics (9), to allow for the introduction and selection of RP4 as a potential mobilizing plasmid. Transfer of plasmids between strains of *E. coli*, together with incompatibility experiments, was carried out by using standard methods and media (10). The strains of *E. coli* K-12 used were J53.2, J62, and CSH52 (9). P incompatibility group plasmids

used were RP4 and R702 (9). Colicin production was tested by a seeded agar overlay method (5).

In an attempt to mobilize the trimethoprim resistance genes in the clinical isolates, J62(RP4) was crossed with each isolate, and selection was made for transfer of RP4. RP4 failed to transfer to 12 of the 50 strains tested. The reasons for this were not investigated further, but could include, among other reasons, restriction of the incoming plasmid DNA by the recipient strain or surface exclusion.

The remaining 38 strains, which now carried RP4, were crossed with J53.2. Mobilization of trimethoprim resistance occurred from 18 of these strains (Table 1). Interestingly, mobilization was only observed from strains received from hospital sources. A further 13 strains transferred RP4 efficiently ($>10^{-5}$ per recipient) but did not mobilize trimethoprim resistance ($<10^{-9}$ per recipient). These 13 strains were considered to carry either chromosomal mutations to trimethoprim resistance (7, 8) or, alternatively, nonconjugative plasmids or defective transposons incapable of being mobilized by RP4. A final group of seven strains showed no transfer of RP4 to J53.2, but each of these strains was found to produce a colicin active against J53.2, which prevented the detection of any transfer events.

The 18 trimethoprim-resistant J53.2 derivatives were crossed with J62, selecting for the transfer of tetracycline resistance (an RP4 marker) and trimethoprim resistance. Six strains showed independent transfer of RP4 and trimethoprim resistance, demonstrating the presence of a separate trimethoprim resistance plasmid. However, subsequent experiments with the transconjugants demonstrated that trimethoprim resistance remained nonconjugative in the absence of RP4. The remaining 12 strains did not

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TABLE 1. Mobilization of trimethoprim resistance from clinical isolates of *E. coli* carrying RP4

Strain no.	Hospital[a]	Frequency of transfer[b]		Transposable resistance(s)[c]
		RP4	Tp^r	
361	City	3.0×10^{-5}	1.5×10^{-7}	
362	City	1.0×10^{-5}	1.0×10^{-7}	TpSm
586	General	8.5×10^{-5}	2.2×10^{-5}	
728	General	1.2×10^{-2}	1.5×10^{-7}	TpSm
842	City	1.5×10^{-3}	6.0×10^{-7}	TpSm
845	City	7.7×10^{-3}	3.4×10^{-6}	TpSm
858	City	1.0×10^{-4}	6.5×10^{-7}	TpSm
902	Basford	1.5×10^{-4}	3.5×10^{-8}	
926	General	2.1×10^{-4}	6.4×10^{-6}	TpSm
933	Newark	1.4×10^{-3}	3.3×10^{-7}	Tp
961	Children's	9.1×10^{-3}	3.8×10^{-6}	TpSm
963	Newark	7.5×10^{-5}	3.4×10^{-8}	Tp
981	Ilkeston	1.4×10^{-2}	1.1×10^{-5}	Tp
995	City	2.7×10^{-4}	1.8×10^{-6}	
997	City	2.8×10^{-5}	1.1×10^{-8}	
1002	Sherwood	2.3×10^{-5}	6.3×10^{-8}	Tp
1004	University	1.3×10^{-6}	1.3×10^{-5}	
1032	Newark	2.6×10^{-2}	5.6×10^{-5}	TpSm

[a] All hospitals are located within a 20-mile (32.18-km) radius of Nottingham.
[b] Transconjugants per recipient cell.
[c] TpSm, Trimethoprim and streptomycin; Tp, trimethoprim.

transfer RP4 independently of trimethoprim resistance, and all resistances were now transferred at the higher frequency previously associated with RP4. Thus, in these 12 strains, the trimethoprim resistance determinant became incorporated as part of RP4 and did not constitute a separate plasmid.

The above incorporation could have occurred by normal recombination processes or as a result of transposition. Normal recombination processes are largely dependent on the *recA* gene product, but transposition is *recA* independent (4). Transconjugants of the *recA* strain CSH52 containing the RP4-Tp^r derivatives were crossed with J53.2(R702), and selection was made for R702 transfer. R702, like RP4, belongs to the P incompatibility group and is unable to coexist stably in the same cell as RP4. From each cross, 100 R702 transconjugants were purified and tested for the presence of RP4. As expected, in every case tested, RP4 had been eliminated. However, a proportion (varying between 1 and 20%) of the transconjugants from each cross was still shown to be trimethoprim resistant, demonstrating that trimethoprim resistance had transposed from RP4 to another replicon. Further experiments similar to those described previously (9) demonstrated that in some cases, trimethoprim resistance had been transposed to the chromosome and in other cases to the incoming R702. Thus, for 12 of the original clinical strains, trimethoprim resistance had been mobilized into *E. coli* K-12 after transposition to RP4.

Each RP4 derivative was tested to see if other resistances from the original clinical strain had also transposed to RP4. Of the 12 plasmids, 8 also conferred streptomycin resistance, and further experiments in which RP4 was used to eliminate R702 carrying a trimethoprim resistance transposon demonstrated that trimethoprim and streptomycin resistances were transposed together as a linked unit. The remaining four RP4 derivatives had not acquired any additional resistances apart from trimethoprim resistance (Table 1).

The majority of trimethoprim resistance transposons previously isolated have encoded linked trimethoprim-streptomycin resistances, and only two transposons, Tn402 (6) and Tn78 (3), that only determine trimethoprim resistance have been reported. Tn402 has been shown to integrate at low frequency into the bacteriophage λ genome (6) but does not integrate into the bacterial chromosome or other resistance plasmids (1). In contrast, the transposons isolated in this study which encoded only trimethoprim resistance were found to transpose to other plasmids and the bacterial chromosome as efficiently as those encoding trimethoprim-streptomycin resistances. They may, therefore, be related to Tn78, which may in turn have evolved from Tn7.

In spite of the limited number of strains investigated in this study, transposons were detected in isolates from five different hospitals (Table 1). It therefore seems that transposable trimethoprim resistance, in the absence of a conjugative trimethoprim resistance plasmid, is probably fairly common in hospitals of the Nottingham area of the United Kingdom.

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