PLASMID-ENCODED, TRIMETHOPRIM-RESISTANT DIHYDROFOLATE REDUCTASES FROM CLINICAL ISOLATES

E.D. Chirnside, J.T. Smith, Microbiology Section, Department of Pharmaceutics, The School of Pharmacy, London WC1N 1AX.

Since 1975 there has been a rapid increase in transferable trimethoprim resistance (Tp^R) among pathogens causing urinary tract infections (UTI) at the Whittington Hospital, London. From 1975-79 the frequency of transferable Tp^R rose within the in-patient population while that of out-patients remained static. But in 1981 pathogens from out-patients exhibiting transferable Tp^R suddenly outnumbered in-patient isolates and this trend continued in 1983 (Chirnside *et al*, 1984).

Bacterial isolates exhibiting Tp^R from patients with UTI attending the Whittington Hospital between 1975-1983 were screened for plasmids encoding Tp^R. In each year studied the sampling period was three months. The Tp^R plasmids were identified by their ability to transfer to *E.coli* K12 J62.2. The dihydrofolate reductases (DHFR's) synthesised in these J62.2 recipients were first purified by ion exchange chromatography, then classified by the isoelectric focusing (IEF) method of Broad and Smith (1982). The plasmid-encoded Tp^R DHFR's exhibited three distinct isoelectric points (pI) on polyacrylamide gels. (pI 6.4 = Type 1; pI 5.5 = Type 2; pI 7.2 = Type 3).

	Dihydrofolate reductase type									
	1		2		3		Unclassified		Totals	
Patient type	IN	OUT	IN	OUT	IN	OUT	IN	OUT	IN	OUT
1975	1	0	2	1	0	1	1	0	4	2
1977	2	2	1	1	2	0	4	0	9	3
1979	9	2	3	0	0	0	0	0	12	2
1981	1	5	2	4	0	0	0	0	3	9
1983	10	17	2	2	0	0	1	0	13	19
Totals	23	26	10	8	2	1	6	0	41	35

The table shows that increasing numbers of type 1 DHFR between 1975 and 1979 mainly accounted for the greater frequency of transferable Tp^R found among in-patient bacteria. But since 1979 the number of type 1 DHFR's rose dramatically among out-patient pathogens and account for the greatest part of the increase in transferable Tp^R in recent years. Over the whole survey type 2 DHFR's have been isolated consistently at low frequencies from both in- and out-patients, whereas the type 3 enzyme was only observed in 1975 and 1977. All the unclassified DHFR's were from in-patient isolates.

Our results at the Whittington Hospital hence show that type 1 DHFR is largely to blame for the increase that occurred with transferable Tp^R, not only from 1975-79 (in-patients), but also from 1981-83 (out-patients). It is known that this type of DHFR can be carried on a transposon (Barth *et al*, 1976).

Trimethoprim is used not only for the treatment of UTI in humans but also is used in intensive livestock husbandry. Such veterinary use of Tp may have been a contributory factor to the sudden recent increase in the incidence of transferable Tp^R amongst out-patients. The predominance of the type 1 DHFR together with increasing numbers of out-patient Tp^R isolates of pathogenic *E.coli* suggests that the spread of transferable Tp^R may now be set to increase even further if transposition of DNA between plasmids within the faecal flora occurs.

Barth, P.T., Datta, N., Hedges, R.W., Grinter, N.J. (1976) J. Bact. 125 : 800-810.
Broad, D.F., Smith, J.T. (1982) Eur. J. Biochem. 125 : 617-622.
Chirnside, E.D., Emmerson, A.M., Smith, J.T. (1984) J.Pharm. Pharmacol. 36 : 56P.