R Factors Conferring Resistance to Trimethoprim but not Sulphonamides

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R factors conferring high-level resistance to trimethoprim and sulphonamides were identified in bacteria causing infections in patients at three London hospitals in 1971 (Fleming, Datta & Grüneberg, 1972). All were members of the W compatibility group and came from the Camden area (Datta & Hedges, 1972).

In April 1972 strains of *Escherichia coli* highly resistant to trimethoprim were isolated from calves at Frant, Kent, which is about 30 miles from the Camden area of London. The calves had been treated with large doses of a trimethoprim-sulphonamide preparation (Fleming, in preparation). The trimethoprim-resistant strains had various patterns of multiple drug resistance (always including resistance to sulphonamides) and from them various R factors were transferred to *E. coli* K12. Trimethoprim resistance was transferred with resistance to streptomycin, separately from resistance to sulphonamides or other drugs. Methods were as described previously (Fleming *et al.* 1972).

An example of the trimethoprim-streptomycin resistance factors (R483) was studied in strains of *Escherichia coli* K12. Methods were as described by Datta & Hedges (1972) and by Coetzee, Datta & Hedges (1972).

Phenotype of trimethoprim-streptomycin R factors

R483 and all other trimethoprim-streptomycin resistance factors tested were *fi*⁻. R483 was therefore tested for compatibility with all known classes of *fi*⁻ R factors.

In all transfers where the recipient was streptomycin-sensitive, exconjugants that had acquired trimethoprim resistance also acquired streptomycin resistance. Thus the two determinants are presumably markers of a single plasmid.

Like W plasmids, which confer resistance to trimethoprim (Fleming *et al.* 1972; Datta & Hedges 1972), R483 determined resistance to > 1000 µg/ml of the drug. As well as streptomycin resistance, R483 conferred resistance to spectinomycin (Hedges, 1972*b*).

Compatibility of R483

The frequency of transfer of R483 from *Escherichia coli* K12 SC16 to J53 with and without R factors of the various compatibility groups is given in Table 1. R483 was compatible with all these, and therefore belonged to a new group, which we designate B.

Although R300 and R483 are compatible (i.e. co-exist stably in K12), the presence of either in a recipient reduces the frequency of transfer of the other (Table 1).

Table 1. *Transfer of R483 to J53 with and without R factors of various compatibility groups*

Donor	Recipient	Compatibility group of resident R factors	Resistances of resident plasmid	Reference	Frequency of transfer	Exconjugant clones tested for presence of each plasmid
sc16 (R483)	J53	—	—	—	1×10^{-3}	—
	J53 (R144)	I	TK	Meynell & Datta (1969)	1×10^{-3}	20/20
	J53 (R45)	N	ATSu	Hedges (1972 <i>a</i>)	1×10^{-3}	20/20
	J53 (RP4)	P	ATK	Datta, Hedges, Shaw, Sykes & Richmond (1972)	1×10^{-3}	20/20
	J53 (R401)	T	AS	Coetzee, Datta & Hedges (1972)	1×10^{-3}	20/20
	J53 (S.a)	W	SCKSu	Hedges & Datta (1971)	4×10^{-4}	20/20
	J53 (R57b)	C	ACSuGk	Witchitz & Chabbert (1972)	1×10^{-3}	20/20
	J53 (R91)	J	K	Datta & Hedges (unpublished)	1×10^{-3}	both present
	J53 (RA1)	A	TSu	Coetzee <i>et al.</i> (1972)	1×10^{-3}	20/20
	J53 (R300)	—	SSu	Hedges & Datta (1971)	1×10^{-3}	20/20
	J53 (R387)	—	SC	Lawn, Meynell, Meynell & Datta (1967)	1×10^{-5}	19/20
	J53 (R6K)	—	AS	Hedges & Datta (1971)	7×10^{-4}	20/20
				Kontomichalou, Mitani & Clowes (1970)	1×10^{-3}	20/20
J53 (R483)	J62	—	—	—	1×10^{-3}	—
J62 (R300)	J62 (R300)	—	SSu	—	1×10^{-5}	20/20, both present
	J53	—	—	—	1×10^{-4}	—
	J53 (R483)	—	STp	—	5×10^{-6}	20/20, both present

sc16, J53 and J62 are nutritionally distinguishable lines of *Escherichia coli* K12.

From each 'double' the two plasmids were transmitted separately.

W plasmids which confer trimethoprim resistance also confer resistance to sulphonamides (Datta & Hedges, 1972). These two synthetic drugs both affect folic acid metabolism. Trimethoprim is always used in conjunction with sulphonamide in human and veterinary medicine and thus any bacterium which acquires trimethoprim resistance is at no selective advantage unless it is also sulphonamide-resistant. However, sulphonamide resistance is fairly common in *Escherichia coli*, and all the calf *E. coli* strains carrying trimethoprim-streptomycin resistance factors also carried other R factors conferring sulphonamide resistance.

Since R483 was compatible with R factors of all the compatibility classes so far described we designate it the prototype of a new class, B. The exclusion of R483 by R300 and vice versa, although the two are compatible, contrasts with previous reports, where exclusion of one plasmid by another has usually been associated with incompatibility (Watanabe, 1969). W plasmids, on the other hand, exert little if any exclusion; the resident plasmid is eliminated by entry of an incompatible replicon (Hedges & Datta, 1971).

Trimethoprim is a newly introduced, synthetic antibacterial drug used in human medicine for 3 to 4 years (Garrod, 1969) and in veterinary medicine for a shorter time. Although bacteria have been exposed to the drug for so short a time, two independent R factor classes have emerged, conferring trimethoprim resistance.

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