DETECTION OF NEW LOW-LEVEL TRIMETHOPRIM RESISTANCE PLASMIDS

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Two recent reports have indicated that not all trimethoprim (Tp) resistance plasmids (R-plasmids) confer high-level resistance to Tp (minimum inhibitory concentration (MIC) > 1000 mg/L). In one of these cases the mechanism has been attributed to the plasmid encoded production of a new Tp resistant dihydrofolate reductase (DHFR) (Fling et al 1982). In the other case it was due to the influence of the host bacterium on the plasmid DHFR activity (Amyes et al 1982).

During a recent survey conducted at the Christian Medical College Hospital in Vellore, India, we isolated a total of 66 self-transmissible Tp R-plasmids from 182 Gram negative urinary pathogens resistant to 10mg/L Tp (Young et al 1985). The Tp R-plasmids had been transferred to the rifampicin resistant Escherichia coli strain J62-2 and selection of Tp resistant transconjugants was made on Oxoid Diagnostic Sensitivity Test Agar (DSTA) containing Tp (10mg/L) and rifampicin (25 mg/L). By re-testing the remaining "non-transferable" strains and selecting on suitably supplemented minimal medium (DM) (Davis & Mingioli 1950) containing the same drugs, a further 36 Tp R-plasmids were identified. Routine characterisation of these latter plasmids by resistance profile revealed that 12 of the J62-2 transconjugant strains grew either weakly or not at all on DSTA containing 10 mg/L Tp. The MIC of Tp for each of these strains was determined on a variety of complex media and suitably supplemented DM minimal medium. The results (Table) show that the MIC of Tp for all 12 strains in DM medium is between 8-30 times higher than in any of the complex media tested.

No. of strains	MIC of Tp on various media (mg/L)					
	DM	DSTA	DSTA-LB	M-H	IA	DM+met+gly+Ad
5	160	20	10	5	5	10
4	160	10	10	5	5	10
1	80	10	10	5	5	10
1	160	20	40	10	10	40
1	320	40	160	40	40	160

DSTA-LB = DSTA + 5% lysed horse blood; M-H = Difco Mueller Hinton agar;
IA = Oxoid Isosensitest agar; met = methionine; gly = glycine; Ad = adenine.

It is known that for Tp to exert a bactericidal response it is necessary for methionine, glycine and a purine derivative to be present in the medium (Amyes and Smith 1974). The MIC of Tp for each organism was re-determined in suitably supplemented DM medium to which methionine, glycine and adenine had been added. The results (Table) show that addition of these supplements resulted in a dramatic reduction in the MIC of Tp for each transconjugant to a level similar to that obained in complex media.
The results demonstrate that some of the Tp R-plasmids we isolated in India are different from those found elsewhere because they not only confer low levels of resistance to Tp, but also are expressed only in certain media. These results suggest that conventional plasmid identification methods, in current use, may fail to detect certain Tp R-plasmids.

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