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NOVEL TRIMETHOPRIM-RESISTANT DIHYDROFOLATE REDUCTASES FROM TWO OUTBREAKS OF *SHIGELLA SONNEI*

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Shigella spp. are the commonest aetiological agent of gastroenteritis in many parts of the world and, as a result of increasing resistance to ampicillin, trimethoprim containing therapy has been used widely to treat such infections. Consequently, alarming increases in trimethoprim resistance in Shigella have been reported from many parts of the world (Elwell & Fling 1989). The most important mechanism of trimethoprim resistance is the plasmid-encoded production of an additional trimethoprim resistant dihydrofolate reductase (DHFR) and a number of these enzymes have been identified and are distinguished by their biochemical profiles (Amyes 1989). Although some studies on trimethoprim resistant Shigella sonnei have demonstrated the presence of the commonly occurring type I DHFR, others have suggested that trimethoprim resistance may result from, as yet, uncharacterised enzymes.

The plasmid-mediated DHFR's responsible for trimethoprim resistance in two outbreaks of Shigella sonnei in the United States have been examined. One outbreak was at a nursing home in East Tennessee and involved 60 patients and several medical personnel. The second was at the Rainbow Family gathering in the Smokey Mountains National Park, North Carolina and is estimated to have involved 6000 people. Preliminary screening with DNA probes representing the commonly occurring DHFR types suggested that the enzymes in each outbreak were different from each other and not of the commonly occurring types. Biochemical characterisation of each enzyme was performed as described by Amyes (1989). This revealed that the two enzymes were indeed different from each other but both showed similarities to the previously isolated type III DHFR (Table 1). Because of the overall biochemical similarity between the three enzymes, isoelectric focusing was used to confirm that these were three distinct DHFR's and they were subsequently named IIIa, IIIb and IIIc.

Table 1. Biochemical properties of the type III plasmid-encoded DHFRs

Enzyme Sub-Type	Tp MIC mgL^{-1}	Tp ID_{50} μM	Methotrexate ID_{50} μM	TD_{50} mins	DHF Km μM	Tp Ki μM	Size Daltons	pI
IIIa	64	2.0	0.02	>12	0.40	0.019	16900	6.10
IIIb	128	2.0	0.02	>12	9.52	0.40	17000	5.34
IIIc	256	3.0	0.007	8	3.12	0.52	22000	5.65

The type III group of enzymes is unusual amongst the plasmid-mediated DHFRs in that they confer only a moderate level of resistance on the host bacterium and are more sensitive to inhibition by trimethoprim than other enzyme types. This may be related to the fact that the type III enzymes have all been isolated from enteric infections; the lower levels of trimethoprim found in the gut than in the urine may allow the survival of strains harbouring these enzymes.

We should like to thank the Wellcome Trust for grant number 16376/1.5 which funded this work.

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