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Plasmid-encoded trimethoprim resistance in salmonellas isolated in Britain between 1970 and 1981

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SUMMARY

Trimethoprim resistance was plasmid-encoded in all trimethoprim-resistant *Salmonella typhimurium* and in the majority of trimethoprim-resistant salmonellas of other serotypes isolated since 1970 from humans and food animals in Britain. In *S. typhimurium*, non-autotransferring plasmids of compatibility group 3 and autotransferring plasmids of group H_2 predominated. The predominance of these plasmid types has resulted from the spread of clones of trimethoprim-resistant strains of phage types 18, 170 and 204c. In other salmonellas, a variety of plasmid compatibilility groups have been identified. Almost all plasmids which conferred resistance to trimethoprim also coded for sulphonamide resistance.

INTRODUCTION

An earlier report described the incidence of trimethoprim resistance in salmonellas which had been isolated in Britain from humans and food animals since 1970 and which had been referred to the Division of Enteric Pathogens (Ward, Rowe & Threlfall, 1982). Resistance to trimethoprim was particularly evident in *Salmonella typhimurium* and by 1981, 6·7% of strains from humans and 14·0% of strains from cattle were trimethoprim-resistant. In other salmonellas, 0·5% of strains isolated in 1981 from humans were trimethoprim-resistant. This paper provides an account of the transferability of trimethoprim resistance in these strains and of the plasmids which specified resistance to trimethoprim.

MATERIALS AND METHODS

Bacterial strains

The source and identification of the trimethoprim-resistant strains have been described previously (Ward *et al.* 1982).

Resistance transfer and plasmid characterization

Strains were tested for the ability to transfer trimethoprim resistance, either directly or by mobilization, to an *Escherichia coli* K12 F^- *lac*$^+$ nalidixic acid-resistant recipient strain. The methods were those of Anderson & Threlfall (1974).

Table 1. *Transfer of trimethoprim resistance*

Serotype	No.*	No. tested	Resistance transfer		Total with transferable trimethoprim resistance
			Directly	Mobilization	
S. typhi	3	3	1	0	1
S. typhimurium					
Human	693	693	243 (35·1)	450 (64·9)	693 (100·0)
Bovine	718	718	647 (90·1)	71 (9·9)	718 (100·0)
Others†	37	37	17 (45·9)	20 (54·1)	37 (100·0)
Other serotypes	101	67	29 (43·3)	19 (28·3)	48 (71·6)

In parentheses: strains with autotransferring or mobilizable trimethoprim resistance as percentage of number tested.

* Number of trimethoprim-resistant strains referred to the Division of Enteric Pathogens.

† Ovine, porcine, avian.

Plasmids which conferred trimethoprim resistance were classified by their incompatibility with autotransferring and non-autotransferring plasmids of the compatibility groups listed by Willshaw *et al.* (1980), and with other non-autotransferring plasmids described by Smith, Humphreys & Anderson, (1974).

RESULTS

Transfer of trimethoprim resistance

Trimethoprim resistance was transferable either directly or by mobilization in one of three resistant strains of *S. typhi*, in all strains of *S. typhimurium* (100%) and in 48 of 67 strains of other salmonella serotypes that were tested (71·6%) (Table 1).

S. typhi

Trimethoprim resistance was directly transferable in one strain of R-type TmCSSu (Tm = trimethoprim, C = chloramphenicol, S = streptomycin, Su = sulphonamides). The plasmid which specified resistance to trimethoprim was of the I complex (Datta, Richards & Datta, 1981); the strain carried a second autotransferring plasmid of the H_2 compatibility group which coded for CSSu. In the two other trimethoprim-resistant strains, trimethoprim resistance was neither transferable nor mobilizable and the levels of resistance were low (8 μg/ml). In contrast, in the strain with plasmid-encoded trimethoprim resistance, the level of resistance was 1000 μg/ml.

S. typhimurium

The plasmids which conferred trimethoprim resistance in strains from humans are shown in Table 2 and those in strains from food animals in Table 3.

Autotransferring trimethoprim resistance plasmids were of the H_2, I_1 and I_2 groups; one plasmid which coded for TmC was not identified. Non-autotransferring plasmids were of two types. Those of one type specified resistance to up to eight drugs including trimethoprim, and were of the F_1*me* compatibility group. Plasmids of the second type coded for resistance to TmSu or Tm alone. These plasmids were

Table 2. *Trimethoprim resistance plasmids in* S. typhimurium *of human origin*

Autotransferring			Non-autotransferring		
Group	Resistances specified	No.	Group	Resistances specified	No.
H_2	TmCSSuT	170	3*	TmSu	382
	TmSSuT	22		Tm	3
	TmC	12		Total	387
	TmACSSuT	1			
	Total	205	F_1me	TmACGKSSuT	46
				TmACKSSuT	7
I_1	TmKS	13		TmACSSuT	5
	TmS	8		TmACGSSuT	2
	TmK	6		TmAGKSSuT	1
	TmSu	3		TmAKSSuT	1
	TmT	1		TmT	1
	Tm	1		Total	63
	Total	32			
I_2	TmS	5			
UC†	TmC	1			
	Grand total	243		Grand total	450

Resistance symbols: A, ampicillin; C, chloramphenicol; G, gentamicin; K, neomycin-kanamycin; S, streptomycin; Su, sulphonamides; T, tetracyclines; Tm, trimethoprim;

* In accordance with scheme of Smith (1975) for the classification of non-autotransferring plasmids.

† Not classified.

Table 3. *Trimethoprim resistance plasmids in* S. typhimurium *from food animals*

	Autotransferring			Non-autotransferring		
Host	Group	Resistances specified	No.	Group	Resistances specified	No.
Cattle	H_2	TmCSSuT	476	3	TmSu	70
		TmSSuT	74		Tm	1
		TmC	11		Total	71
		Total	561			
	I_1	TmKS	31			
		TmS	21			
		TmSu	2			
		TmK	1			
		Tm	1			
		Total	56			
	I_2	TmS	30			
		Grand Total	647		Grand Total	71
Pigs, sheep, poultry	H_2	TmCSSuT	15	3	TmSu	18
		TmC	1		Tm	2
		Total	16		Total	20
	I_1	TmS	1			
		Grand Total	17		Grand Total	20

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Table 4. *Trimethoprim resistance plasmids in other serotypes*

Serotype*		Autotransferring			Non-autotransferring		
		Group	Resistances specified	No.	Group	Resistances specified	No.
S. agona	(2)	—	—	—	—	TmT†	1
		—	—	—	—	Tm†	1
S. anatum	(1)	I_2	TmS	1	—	—	—
S. bareilly	(1)	C	TmACKSu	1	—	—	—
S. brandenburg	(1)	I_2	TmS	1	—	—	—
S. chicago	(2)	H_2	TmASSuT	2	—	—	—
S. derby	(1)	H_2	TmSuT	1	—	—	—
S. enteritidis	(1)	I_2	TmS	1	—	—	—
S. hadar	(1)	—	—	—	3	TmSu	1
S. heidelberg	(8)	H_2	TmCSSuT	2	H_2	TmCSSuT	1
		H_2	TmACKSSuT	1	—	—	—
		H_2	TmACGKSSuT	1	—	—	—
		I_1	TmKS	1	—	—	—
		I_1	TmS	1	—	—	—
		I_1	Tm	1	—	—	—
S. infantis	(1)	H_2	TmCSSuT	1	—	—	—
S. mbandaka	(1)	H_2	TmSSuT	1	—	—	—
S. meleagridis	(5)	N	TmASSuT	5	—	—	—
S. montevideo	(3)	C	TmCSuT	1	—	—	—
		C	TmACKSuT	1	—	—	—
		I_1	TmKS	1	—	—	—
S. muenchen	(10)	—		—	F_1me	TmACKSSuT	9
					F_1me	TmAGKSSu	1
S. oranienburg	(2)	F_1me	TmACGKSSuT	1	—	—	—
		I_1	TmSu	1	—	—	—
S. panama	(1)	H_2	TmCSSuT	1	—	—	—
S. saintpaul	(2)	H_2	TmCSSuT	1	H_2	TmCSSuT	1
S. virchow	(1)	N	TmASSuT	1	—	—	—
S. wien	(4)	—	—	—	F_1me	TmACKSSuT	1
		—	—	—	F_1me	TmACSSuT	2
		—	—	—	F_1me	TmACGKSSu	1

* Number of strains in parentheses.
† Compatible with standard non-autotransferring plasmids.

incompatible with the tetracycline resistance determinant NTP 5 (Smith *et al.* 1974) and were therefore assigned to group 3 of non-autotransferring plasmids in accordance with the scheme of Smith (1975). Non-autotransferring TmSu plasmids were identified in strains from humans and food animals, whereas F_1me plasmids were found only in strains from humans.

Other serotypes

Sixty-seven trimethoprim-resistant strains of serotypes other than *S. typhi* and *S. typhimurium* were tested. Trimethoprim resistance was directly transferable in 29 strains of 15 serotypes (43.3%) and was mobilizable in a further 19 strains of 6 serotypes (28·4%) (Table 1).

The plasmids which coded for trimethoprim resistance were characterized and the results are summarized in Table 4. Autotransferring plasmids were of the C, F_1me, H_2, I_1, I_2 and N groups. Non-autotransferring F_1me plasmids were identified in strains of *S. muenchen* and *S. wien*, a group 3 resistance determinant which coded

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for TmSu was identified in a strain of *S. hadar*, and strains of *S. saint paul* and *S. heidelberg* carried transfer-defective H_2 plasmids which coded for TmCSSuT. Two non-autotransferring plasmids in strains of *S. agona* were not classified.

DISCUSSION

These studies have shown that trimethoprim resistance was plasmid-encoded in all trimethoprim-resistant *S. typhimurium* and in the majority of trimethoprim-resistant strains of other salmonella serotypes that have been isolated in Britain since 1970. This constrasts with previous findings with *E. coli*, *Klebsiella aerogenes*, *Proteus mirabilis* and *Enterobacter spp* isolated from humans (Brumfitt, Hamilton-Miller & Grey, 1977; Towner *et al.* 1978), although an increase in plasmid-specified resistance in these organisms has recently been reported (Towner *et al.* 1980). Furthermore, plasmids which coded for trimethoprim resistance in salmonellas were, in general of different compatibility groups than those in coliforms, where plasmids of the W, F_{II}, I_2 and B groups have predominated. (Grey, Hamilton-Miller & Brumfitt, 1979; Towner, 1979).

In *S. typhi*, the one strain with high-level transferable trimethoprim resistance had acquired resistance whilst the patient was being treated with co-trimoxazole (Datta *et al.* 1981). The low level of resistance in the two other trimethoprim-resistant strains was probably chromosomal.

In both human and bovine trimethoprim-resistant *S. typhimurium*, the predominance of non-autotransferring plasmids of group 3 and autotransferring plasmids of group H_2 has resulted from the spread of trimethoprim-resistant clones of phage types 18, 170 and 204c (Ward *et al.* 1982). Strains of types 18 and 170 carried non-autotransferring plasmids of group 3 which coded for TmSu whilst type 204c strains carried H_2 plasmids which transferred TmCSSuT (T = tetracyclines) or TmSSuT (Threlfall *et al.* 1980). Group I_1 plasmids were distributed amongst various phage types but I_2 plasmids were found only in related strains of phage types 49, 204, 204a and 193 (Threlfall, Ward & Rowe 1978; Threlfall, 1982). Non-autotansferring F_I*me* plasmids were found exclusively in strains from humans. These strains were of phage types 208, 66/122 or untypable and were isolated from persons infected in the Middle East or South East Asia, or from persons of Asian origin (Rowe *et al.* 1980; Frost *et al.* 1982).

The incompatibility between the tetracycline resistance determinant NTP 5 and the non-autotransferring TmSu plasmids was particularly interesting. NTP 5 was identified in a strain of *S. typhimurium* phage type 49 isolated in 1969 and, until these studies, has proved to be compatible with all other non-autotransferring plasmids we have tested. NTP5 is a multiple-copy plasmid of molecular weight (MW) $6{\cdot}5 \times 10^6$ (Smith *et al.* 1974). Studies are in progress to determine the MW of the non-autotransferring TmSu plasmids and the number of copies of these plasmids per chromosome.

A variety of plasmid types were encountered in trimethoprim-resistant salmonellas of other serotypes. Non-autotransferring F_I*me* plasmids were found in strains of *S. muenchen* and *S. wien* and in several cases the patient had been infected in areas where F_I*me* plasmids are widely distributed in number of salmonella serotypes (Rowe *et al.* 1980). Of the other plasmid types, I_1, I_2, H_2 and group 3 plasmids have been observed in trimethoprim-resistant *S. typhimurium*

and group C plasmids which coded for trimethoprim resistance have been identified in *S. bareilly* from outbreaks in India (DEP, unpublished observations).

Until recently, trimethoprim has been available only in trimethoprim–sulphonamides combinations. A consequence of the use of such combinations has been that the majority of plasmids which conferred resistance to trimethoprim also coded for sulphonamide resistance. Trimethoprim-containing products have now been released for use without the sulphonamide component and it is possible that the use of this drug alone will result in a proliferation of plasmids which confer resistance to trimethoprim but not to sulphonamides. This may be avoided by restricting the use of trimethoprim alone for prophylaxis and, in the treatment of salmonella infections, by using this drug only when the strains spread extra-intestinally.

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