

Acquisition of a drug resistance plasmid converts *Salmonella enteritidis* phage type 4 to phage type 24

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SUMMARY

Salmonella enteritidis accounted for 55% of the 27 478 salmonellae isolated from humans in England and Wales during 1988. Within this serotype phage type 24 has increased from 24 isolations in 1987 to 201 in 1988. The high frequency of drug resistance in this phage type has been shown to be due to the presence of plasmids belonging to Inc N and coding for resistance to a range of antimicrobial drugs among which resistance to ampicillin, streptomycin, tetracycline (AST) and T predominate. These plasmids are phage-type determining and convert strains of phage type 4 to phage type 24.

INTRODUCTION

Salmonella enteritidis is the most common salmonella serotype isolated from humans in England and Wales. In 1988 the Division of Enteric Pathogens (DEP) received 27 478 salmonellae isolated from humans, of which 15 427 were *S. enteritidis*. *S. enteritidis* can be subdivided using a phage-typing scheme developed in the DEP (1). Two hundred and one of the strains isolated in 1988 belonged to phage type 24. This type was first identified in 1987 when 24 strains were isolated.

Resistance to antimicrobial drugs is rare in *S. enteritidis*. In 1987 only 735 (10.7%) of 6858 *S. enteritidis* isolated in England and Wales were drug-resistant. By comparison, 211 (93.7%) of the 225 *S. enteritidis* phage-type 24 strains isolated during 1987 and 1988 were resistant to one or more drugs. Changes in phage type have been associated with the acquisition of drug resistance plasmids in *S. typhimurium* (2) and in *S. typhi* (3). The plasmids present in *S. enteritidis* phage-type 24 strains have been characterized and the relationship between phage type and drug resistance has been investigated to ascertain whether a similar phenomenon is occurring in *S. enteritidis*.

MATERIALS AND METHODS

Bacterial strains

All *S. enteritidis* strains referred to the Division of Enteric Pathogens were phage typed as described by Ward and colleagues (1). Phage type 24 strains were tested for resistance to the following antimicrobial drugs (4); ampicillin (A),

Table 1. *S. enteritidis* phage type 24. Drug resistance and foreign travel

Year of isolation	Foreign travel	Total	R-type			
			Sensitive	T	AST	Other
1987	Yes	12	0	9	0	3
	No*	12	5	6	0	1
1988	Yes	17	0	13	3	1
	No*	179	4	14	132	29

* Includes patients where no information was available.

chloramphenicol (C), furazolidone (Fu), gentamicin (G), kanamycin (K), spectinomycin (Sp), streptomycin (S), sulphonamides (Su), tetracyclines (T), trimethoprim (Tm) and nalidixic acid (Nx).

Isolation of plasmid DNA

Plasmid DNA was isolated using the method of Kado and Liu (5).

Plasmid characterization

Plasmids were transferred to standard strains of *Escherichia coli* K12 (DEP ref. 14R525) and *S. enteritidis* phage type 4 (DEP ref. E2187) and tested for incompatibility with plasmids of a range of incompatibility groups (4).

Recipient ability

Standard plasmids from a number of incompatibility groups were transferred from *E. coli* K12 to *S. enteritidis* phage type 4 (DEP ref. E2189) and *S. typhimurium* phage type 36 (DEP ref. 19R688). The recipient strains were drug-sensitive and each carried a serotype specific plasmid, 38×10^6 for *S. enteritidis* and 60×10^6 for *S. typhimurium* (6). All crosses were incubated overnight at 37 °C and serial dilutions plated on MacConkey agar plates containing appropriate antibiotics and colicin E₂ to counterselect against the *E. coli* K12 donor.

RESULTS

Drug resistance and epidemiology

The 24 strains of *S. enteritidis* phage type 24 isolated during 1987 were all isolated from humans. Nineteen strains were drug-resistant (Table 1). Fifteen were resistant only to tetracyclines of which nine were isolated from patients giving a history of recent travel to the Canary Islands. A further three strains were resistant to TFu, two of which were from patients recently returned from the Canary Islands. The 18 strains resistant to tetracyclines had an MIC exceeding 1000 mg/l. One strain, resistant to ASFu, was from a patient returning from Spain.

In 1988, 201 strains of *S. enteritidis* phage type 24 were isolated in England and Wales of which 196 were from humans. No strains were isolated during January or February and the number of isolations was highest during June to October

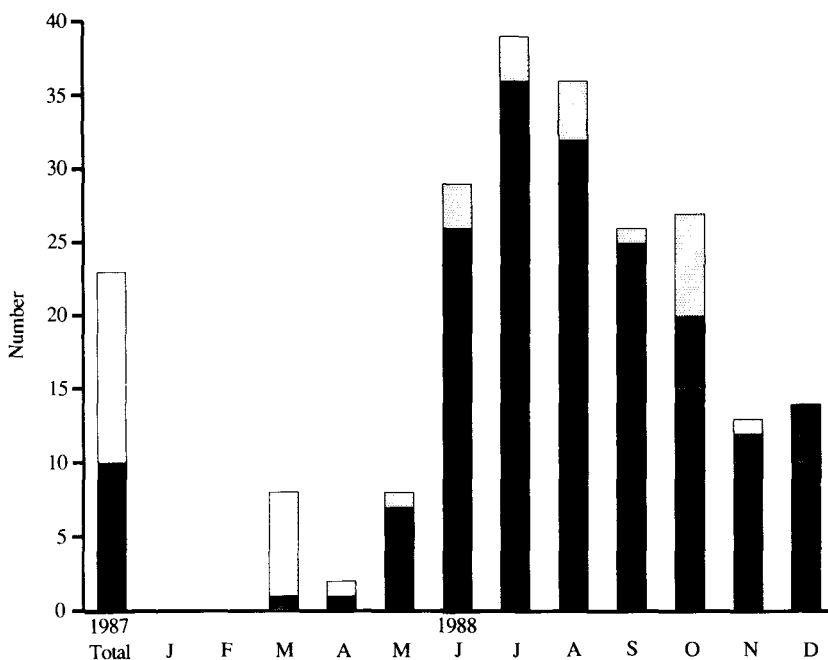


Fig. 1. *S. enteritidis* phage type 24 isolated in England and Wales. R-type: □, T; ▨, other R-types; ■, AST.

(Fig. 1). One hundred and ninety-two strains were drug-resistant; of these, 140 isolated from May onwards, were resistant to AST with MIC's for each drug exceeding 1000 mg/l. The frequencies of the other resistance patterns are given in Table 2.

Thirteen of the 27 tetracycline resistant strains were from patients recently returned from abroad; 12 from the Canary Islands or mainland Spain. Five of these strains were isolated in March from passengers returning on the same flight from Tenerife. Six strains, resistant to ASuTm, were from a hospital outbreak in England. The remainder, including all strains of R-type AST, were isolated from apparently sporadic infections although there were a number of small family outbreaks.

During 1988, three phage type 24 strains were isolated from poultry, one from a dog and one from surface water. All were resistant to AST.

Plasmid profiles and plasmid characterization

Sixty-four strains were screened for plasmid DNA (Table 2). All of these carried a 38×10^6 plasmid which corresponds to the plasmid found in the majority of *S. enteritidis* strains (6). All but one drug resistant strain carried a plasmid of approximately 25×10^6 . This plasmid was transferable and encoded resistance to T, AST, ASuTm, ASuT, AS or AKS corresponding with the R-type of the parent strains. In all cases the resistance plasmids were incompatible with standard plasmids of incompatibility group N (Inc N). Two strains, resistant to ASSuT,

Table 2. *S. enteritidis* phage type 24. Plasmid profiles

Year of isolation	R-type*	Number tested	Plasmid profile molecular size $\times 10^6$					Number
1987	AST	0	—	—	—	—	—	—
	T	2	—	38	25	—	—	1
			56	38	25	—	—	1
	TFu	1	56	38	25	—	—	1
	ASFu	0	—	—	—	—	—	—
	Sensitive	1	—	38	—	—	—	1
Total 1987		4						
1988	AST	22	—	38	25	—	—	22
	T	16	—	38	25	—	—	8
			56	38	25	—	—	8
	ASTFu	2	—	38	25	—	—	2
	ASuTm	3	—	38	25	—	—	3
	ASuT	3	—	38	25	—	—	2
			56	38	25	—	—	1
	AS	2	—	38	25	—	—	1
			—	38	25	2	1	1
	ASSuTTm	2	56	38	25	—	4	1
				38	25	—	4	1
	ASSuT	1	—	38	25	—	—	1
	AKS	1	—	38	25	—	—	1
	ASu	1	—	38	25	—	—	1
	A	1	60	38	—	2	1	1
	Sensitive	2	56	38	25	—	—	1
		—	38	25	—	—	1	
Total 1988		56						

* Drug resistances: A, ampicillin; Fu, furazolidone; K, kanamycin; S, streptomycin; Su, sulphonamides; T, tetracyclines; Tm, trimethoprim.

carried a 4×10^6 SuTm plasmid in addition to the AST Inc N plasmid. The remaining strain carried an Inc I₁ plasmid of 60×10^6 coding for resistance to ampicillin. Thirteen strains carried a 56×10^6 cryptic plasmid. Six of these were from patients recently returned from the Canary Islands.

Two strains, resistant to AS and A respectively, each had two smaller plasmids, 2×10^6 and 1×10^6 , similar in size to those found in the type strain of phage type 24 (7).

The two drug-sensitive strains tested both carried the 38×10^6 plasmid and a 25×10^6 plasmid. One strain also carried a 56×10^6 cryptic plasmid.

Phage type determination

Representative Inc N plasmids encoding the various resistance patterns were transferred to *E. coli* K12 (14R525) and thence to *S. enteritidis* phage type 4 (E2187). The drug-resistant transconjugant was shown to have been converted to phage type 24. Similarly spontaneous segregants of phage type 24 strains which had lost the Inc N plasmid had reverted to phage type 4. Spontaneous phage type 4 segregants from the two drug-sensitive strains tested were also shown to have lost the 25×10^6 plasmid. Thus this plasmid was probably a variant of the Inc N

Table 3. *S. enteritidis* recipient ability

Plasmid			Recipient	
Inc group	Plasmid ref. no.	Resistance	<i>S. typhimurium</i> 36	<i>S. enteritidis</i> 4
B	TP113	K	5×10^{-2}	5×10^{-4}
		T	10^{-4}	10^{-4}
F _{1me}	TP180	K	5×10^{-5}	NTD
	TP160	T	5×10^{-6}	NTD
I ₁	TP110	K	10^{-1}	10^{-4}
	TP189	CT	10^{-1}	5×10^{-3}
I ₂	TP114	K	10^{-2}	5×10^{-5}
N	TP209	T	NTD	5×10^{-4}
		AK	5×10^{-3}	5×10^{-2}
		T	NTD	10^{-6}
		AST	NTD	10^{-7}
	ex P112355*	T	NTD	10^{-6}
	ex P128978*	AST	NTD	10^{-7}

* *S. enteritidis* strains isolated during 1988.
 NTD, no transfer detected.

plasmid present in the drug resistant strains. The ampicillin resistant Inc I₁ plasmid showed no corresponding change in phage type.

Recipient ability

The recipient abilities of *S. typhimurium* and *S. enteritidis* for plasmids of incompatibility groups commonly isolated from enterobacteria in the UK were compared. The results (Table 2) showed that, in most experiments, the transfer frequency of plasmids belonging to incompatibility groups B, I₁ and I₂ was higher when the recipient was *S. typhimurium* than *S. enteritidis*. Inc F_{1me} plasmids did not transfer to *S. enteritidis*.

The two Inc N plasmids originally isolated from *S. typhimurium* strains, transferred to *S. enteritidis* at a higher frequency than to *S. typhimurium*. In contrast, the two Inc N plasmids from *S. enteritidis* phage-type 24 strains transferred at a low frequency to *S. enteritidis* but did not transfer to *S. typhimurium*.

DISCUSSION

In 1987, *S. enteritidis* phage type 24 was infrequently isolated in England and Wales. The strains were predominantly resistant to tetracyclines alone and 12 of 24 strains were isolated from travellers returning from the Canary Islands or Spain. During 1988 this phage type became more prevalent and 97% of the strains were resistant to antimicrobial drugs. The two most common resistance patterns were T and AST. Although in 1988 strains resistant to tetracyclines alone continued to be isolated from people returning from the Canary Islands, 137 of 140 strains of R-type AST, which first appeared in May, were from patients giving no history of recent foreign travel. The three isolates from poultry were also AST resistant and were indistinguishable from the human isolates.

All resistances except Fu were transferable and encoded by plasmids of Inc N.

These plasmids were phage-type determining, converting phage type 4 to type 24. The evidence suggests that drug-resistant phage type 24 strains have been derived from phage type 4 by the acquisition of the Inc N plasmids. Although phage type 24 strains of R-type AST may represent a single clone, the variety of other R-types isolated during 1988 suggests that the conversion of phage type 4 to type 24 was not a unique event. Comparison of the relative transfer frequencies of plasmids commonly encountered in salmonellae showed that, with the exception of Inc N plasmids, *S. enteritidis* is a poor plasmid recipient compared with *S. typhimurium*. The low transfer frequency of resistance plasmids from *S. typhimurium* to *S. enteritidis* is compatible with a similar observation of Anderson & Threlfall (8) who considered that *S. enteritidis* carries a specific restriction system which destroys incoming DNA. This could explain the relative rarity of drug resistance in this serotype.

This study suggests that the increase of human infections with *S. enteritidis* phage type 24 in 1988 should be seen as part of the overall epidemic spread of *S. enteritidis* phage type 4.

Note added in proof

In the first 6 months of 1989, 341 strains of *S. enteritidis* phage type 24 have been isolated from humans, of which 294 (86%) are resistant to AST. During the same period 11 *S. enteritidis* phage type 24 strains of R-type AST have been isolated from chickens, 2 from eggs, and 1 from a turkey. There have also been 3 strains from bovines and 1 from a badger.

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