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Clonal distribution of resistance plasmid-carrying *Salmonella typhimurium*, mainly in the Middle East

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SUMMARY

Strains of *Salmonella typhimurium* of predominantly Middle Eastern origin, but distributed from England to India, were found to carry at least three types of resistance plasmid. The most important was initially identified as an F_I plasmid by compatibility tests, but differs from the F factor on the one hand and the F_I factors R162 and ColV on the other. The three groups of F_I plasmids can be distinguished by their compatibility reactions with the MP10 plasmid of *S. typhimurium* (Smith, Humphreys, Grindley, Grindley & Anderson, 1973) and group H_1 factors: the F factor is unilaterally incompatible with group H_1 (Smith, Grindley, Humphreys & Anderson, 1973; Anderson, 1975*b*); the F_I factors are compatible with MP10 and group H_1; and F_I*me* factors are incompatible with MP10 but compatible with H_1. The majority of *S. typhimurium* cultures belonged to phage type 208; most of those that did not, belonged to types related to 208. Only a minority of their F_I*me* plasmids were autotransferring. The remainder were mobilizable by F-like plasmids, and by group H_1 and H_2 factors, but not by the *fi*$^-$ I_1 factor Δ, or by plasmids of the I_2, B, P, W, N and *com* 7 groups. The compatibility reactions of the autotransferring F_I*me* plasmids were identical with those of the non-transferring members of the group, and both were large, single-copy plasmids.

The *S. typhimurium* strains of this series carried A or AK, and SSu resistance determinants: small, probably multicopy, non-transferring plasmids similar to those originally described in phage type 29 of *S. typhimurium* (Anderson & Lewis, 1965*b*).

These *S. typhimurium* cultures probably represent a clone of wide geographical distribution. The accurate epidemiological study of such clonal outbreaks requires, in addition to phage typing, precise identification of the plasmids harboured by the epidemic strains, and may have to be carried to the molecular level.

F_I*me* plasmids were identified in other drug-resistant salmonellas, notably in a strain of *S. wien* which caused large outbreaks of mainly paediatric infection in Algeria, and also spread to Britain. An F_I*me* plasmid was found in *S. typhi* phage type 44 from Algeria, in which the phage-restricting properties of the plasmid are responsible for the specificity of the type.

Table 1. *Standard bacterial strains*

ERL No.	Description	Plasmids carried	Compatibility group
1R713	*Escherichia coli* K12 F⁻ *lac*⁺ prototrophic	—	.
14R525	1R713 Nal^r*	—	.
27R207	K12 HfrH *lac*⁺ Nal^r	Integrated F	F_I
31R892	K12 F⁺ *lac*⁺ Nal^r	F	F_I
42R500	*Salmonella typhimurium* phage type 36	—	.
Type A	*S. typhi* Vi-type A	—	.
1R380	K12 F⁻ *lac*⁺ (Δ)	Δ	I_1
RT641	*S. typhimurium* type 6 (Δ)	Δ	I_1
18R951	K12 F⁻ *lac*⁻ Str^r† (X)	X	F_{II}
20R770	K12 F⁻ *lac*⁺ (T-Δ *drp*)	T-Δ *drp*	I_1

Δ: *fi*⁻ Group I_1 transfer factor (Anderson & Lewis, 1965*b*).

X: *fi*⁺ Group F_{II} transfer factor (Anderson & Lewis, 1965*b*; Anderson, Pitton & Mayhew, 1968).

T-Δ *drp*: derepressed mutant of the Group I_1 R factor T-Δ (Anderson & Lewis, 1965*b*; Anderson, 1968*b*; Grindley & Anderson, 1971).

* Nalidixic acid-resistant mutant of 1R713.

† Chromosomal resistance to streptomycin.

INTRODUCTION

In a previous communication we outlined compatibility relations of F_I plasmids, both within the F_I group, and with plasmids of other groups (Threlfall, Carr & Anderson, 1976). With the exception of the F factor itself, these plasmids were initially identified in salmonellas: principally *S. typhimurium*, but also in *S. typhi* and *S. wien*. The observations summarized by Threlfall *et al.* (1976) have been extended, and the present article describes these interactions in detail. Special emphasis will be concentrated on the F_I*me* group, so designated because it has widespread Middle Eastern associations, though it has been found in North Africa (Mered *et al.* 1970), France (Le Minor, 1972) and Britain. This group consists of *fi*⁺ plasmids, most of which are non-autotransferring. Those that transfer are incompatible with those that do not, and establish their membership of the F_I groups by incompatibility with the F factor and by stimulating their carrier strains to produce F fimbriae, as shown by their ability to support multiplication of F-specific phages. Other compatibility reactions, to be described in this article, distinguish F_I plasmids from each other. Because they are incompatible with the autotransferring members of the group and with F, the non-transferring members have been allotted to the F_I*me* group, despite the fact that they do not produce F fimbriae.

MATERIALS AND METHODS

Bacterial strains

The standard strains used for the compatibility and other studies are shown in Tables 1 and 2.

The wild strains of salmonellas, with the exception of one bovine culture of *S. typhimurium* from Canada, were isolated in man: their countries of origin are indicated in Tables 3 and 8 (for *S. typhimurium*) and 9 (for other salmonellas).

Table 2. *Autotransferring and non-autotransferring plasmids used in compatibility tests*

Plasmid designation	Compatibility group	Resistance markers	Source	Reference
F	F_I	—	*E. coli* K12	Hayes (1952)
F-*lac*	F_I	—	*E. coli* K12	Jacob & Adelberg (1959)
F-*lac*-T	F_I	T	*	Anderson & Smith (1972*a*)
F-T	F_I	T	†	Anderson (1975*a*)
ColV2	F_I	—	*E. coli* K94	Gratia (1925)
R162	F_I	ACSSuT	*S. typhi* Vi-type E13, (France, 1974)	Chabbert & Gerbaud (1974)
TP160	F_Ime (autotransferring)	ACKSSuT	*S. typhi* Vi-type 44, 1T5626 (Algeria, 1974)	Anderson (1975*b*)
TP181	F_Ime (autotransferring)	ACKSSuT	*S. typhimurium* type 208, 15M3557 (Iran, 1975)	Threlfall *et al.* (1976)
TP187	F_Ime (autotransferring)	ACKSSuT	*S. wien* 77 (England, 1974)	See text (McConnell *et al.*, unpublished work)
NTP101	F_Ime (non-autotransferring)	ACSSuT	*S. typhimurium* type 208, 14M6407 (England, 1974)	Threlfall *et al.* (1976)
$MP10_{36}$	MP10	—	*S. typhimurium* type 36, RT576 (England, 1964)	Anderson & Smith (1972*a*)
fi⁻K‡ (= K)	MP10	K	*S. typhimurium type* 29, 5M4136 (England, 1965)	Anderson, Pitton & Mayhew (1968); Smith *et al.* (1974)
A*	MP10	A	§	Smith, Humphreys *et al.* (1973)
TP123	H_1	CSSuT	*S. typhi* 1T4739 (Mexico, 1972)	Anderson & Smith (1972*b*); Grindley *et al.* (1972)
NTP1	Group 1	A	*S. typhimurium* type 29, RT1 (England, 1964)	Anderson & Lewis (1965*a*, *b*); Smith *et al.* (1974)
NTP2	Group 2	SSu	*S. typhimurium* type 29, RT1 (England, 1964)	Anderson & Lewis (1965*a*, *b*); Smith *et al.* (1974)
NTP3	Group 2	ASu	‖	Anderson, Kelemen *et al.* (1968); Smith *et al.* (1974)

Drug resistance symbols: A, ampicillin; C. chloramphenicol; K, neomycin–kanamycin; S, streptomycin; Su, sulphonamides; T, tetracyclines.

* Recombinant between F-*lac* and the tetracycline resistance marker of T-Δ (Anderson & Lewis, 1965*b*; Anderson, 1968*b*).

† F-T was derived from the F-*lac*-T hybrid plasmid by loss or inactivation of the *lac* operon.

‡ Spontaneous *fi*⁻ mutant of *fi*⁺K, a recombinant between K and MP10, identified in *S. typhimurium* 5M4136 (Anderson, Pitton & Mayhew, 1968).

§ Spontaneous recombinant between the A translocon of NTP3 and MP10.

‖ Made in the laboratory by u.v. irradiation of strain RT1 of *S. typhimurium* (Anderson, Kelemen *et al.* 1968).

Phage-typing

This was carried out in *S. typhi* by the method of Craigie & Felix (1947), review: Anderson & Williams (1956); and in *S. typhimurium* by the methods of Felix & Callow (1943) and Callow (1959); review: Anderson (1964).

Media

The nutrient media used throughout were based on 2% Bacto dehydrated nutrient broth (Difco Laboratories). To this was added 1·3% New Zealand powdered agar (Davis) for the preparation of nutrient agar plates.

Oxoid MacConkey agar No. 3 was routinely employed as a differential medium to distinguish between Lac^+ and Lac^- strains.

Selection was exercised where necessary with the following concentrations of antibacterial drugs:

Drugs	Concentrations	Drugs	Concentrations
Ampicillin	100 μg/ml	Tetracyclines	10 μg/ml
Chloramphenicol	40 μg/ml	Sulphathiazole	100 μg/ml
Kanamycin	20 μg/ml	Trimethoprim	2 μg/ml
Gentamicin	20 μg/ml	Nalidixic acid	40 μg/ml
Streptomycin	20 μg/ml		

Tests for drug resistance (R-typing) and minimal inhibitory concentration (MIC)

These were carried out by the techniques described by Anderson & Threlfall (1974).

Bacterial crosses

The method of Anderson & Lewis (1965*a*) was used for crosses, which were mostly of about 18 h (overnight) duration. When the crosses were of short duration, 1 or 2 h, the ratio of donor to recipient cultures was 1:10.

Triparental crosses for plasmid mobilization were performed by the method of Anderson (1965). The Δ factor, which belongs to compatibility group I_1, and the X factor (Group F_{II}), were routinely used in mobilization tests (Anderson & Threlfall, 1974), but other transfer factors were introduced as necessary.

Sex-specific phage propagation

Plasmids were examined for their ability to stimulate production of F or I sex fimbriae by investigating the capacity of their carrier strains to support growth of the F-specific phages μ2 (Dettori, Maccacaro & Piccinin, 1961) and fd (Marvin & Hoffman-Berling, 1963), and the I-specific phage If1 (Meynell & Lawn, 1968). The method employed was that of Grindley & Anderson (1971).

Compatibility tests

Compatibility tests between pairs of plasmids were carried out by the method of Grindley, Grindley & Anderson (1972). Segregation resulting from incompatibility usually yielded only 10–50% of colonies carrying both plasmids, whereas the rate of segregation of compatible plasmids was no higher than that of their spontaneous loss from strains carrying the plasmids separately. Residual hybrids carrying incompatible plasmids continued to segregate.

RESULTS

Our findings are summarized in Tables 3–9.

Table 3 shows the distribution and properties of F_I*me* plasmids isolated from *S. typhimurium* of Middle Eastern origin. The majority of the Iranian cultures, and all the Israeli cultures, were isolated in their respective countries, mostly from severe paediatric infections. All other strains were isolated in Britain from persons infected in the countries concerned, and were sent to the Enteric Reference Laboratory for phage-typing.

The strains shown under Britain are specially interesting. They were isolated from a small but protracted outbreak of *S. typhimurium* infection in adults and children in a Burns Unit in Birmingham. Five infections, four of which were symptomless, occurred between November and December 1974; seven infections, two symptomless, between March and April 1975; and one infection with symptoms in November 1975. This outbreak provided our first examples of F_I*me* plasmids in November 1974. Symptoms, when they occurred, were those of mild enteritis. Epidemiological studies revealed that the outbreak started after admission to the unit of a severely scalded patient who had been flown as an emergency case from Tehran in November 1974. He was a symptomless excreter of the epidemic strain of *S. typhimurium*. It was reasonably concluded in retrospect that he was the index case: the outbreak strain was therefore imported from Iran, and shared the Middle Eastern origin of the other *S. typhimurium* strains studied. It is interesting that it persisted in the unit for a year, though no cases with symptoms occurred between April and November 1975. There was no evidence that the strain had been introduced more than once, that is, in November 1974.

Resistance spectra (R-types) of resistant S. typhimurium *strains*

These are shown in column 5 of Table 3. The commonest R-type was ACKSSuT, but K was not always present, and other resistances identified were Fu, Nx, G and Tm. Fu and Nx are probably of chromosomal origin, but G and Tm are plasmid-coded. There is further discussion of R-types in the following section.

Resistance transfer

Direct

Autotransferring F_I*me* plasmids were identified principally in cultures from Iran, either in patients infected there and yielding *S. typhimurium* on their return to Britain, three in 1970, one in 1973 and one in 1976; or isolated in Iran, mainly from severe paediatric infections. Of the latter group, 44 of 113 strains transferred their F_I*me* plasmids directly. Direct resistance transfer was also found in one culture isolated in Britain from a person infected in Turkey, and one from an infection acquired in an unidentified place between Pakistan and Turkey. Most directly-transferring F_I*me* plasmids coded for the R-type ACKSSuT (see Tables 3 and 4). However, considerable variation from this pattern was found, especially in the Iranian cultures, as Table 4 shows.

All the autotransferring F_I*me* resistance plasmids formed Class 1 transfer systems, that is, the transfer factor and the resistance linkage group(s) were

Table 3. *Drug resistance transfer in* Salmonella typhimurium *of mainly Middle Eastern origin, 1969–76, Britain, India, Iran, Iraq, Israel, Jordan, Kuwait, Syria, Turkey*

Country of origin	Number of cultures	Year of isolation	*Salmonella typhimurium*		Drug resistance transfer			
						By mobilization†		
			Phage type	R-type*	Directly	F_1me	Group 1	Group 2
Britain‡	13	1974–5	208	ACKSSuT	—	ACSSuT	A	SSu
India	3	1974	208 (1)	ACKSSuTNx	—	ACSSuT	AK	SSu
		1975	170 (1)	AKSSu	—		AK	SSu
			208 (1)	ACKSSuT	—	CT	AK	SSu
Iran	5	1970	208 (3)	ACKSSuT (2)	ACKSSuT AK§ SSu§	.	.	.
				ACKSSuT (1)	—	ACSSuT	A	SSu
		1973	208 (1)	ACKSSuT	ACKSSuT AK§ SSu§	.	.	.
		1976	Untypable (1)	ACKSSuTTmFu	—	ACSSuT	AK	SSu‖
	125¶	1969–74	208 (91)	23 R-types	44 of 113 Iranian cultures (38·9%) transferred F_1me plasmids directly. These also transferred A or AK and SSu in a Class 2 relationship. Non-transferring plasmids were mobilizable as in strains from elsewhere			
			Untypable (21)					
			170 (12)					
			24 (1)					
Iraq	2	1974	208	ACKSSuTFu	—	ACSSuT		SSu
				ACKSSuTFu	—	ACSSuT	AK	SSu
Israel	44**	1971–3	208 (8)	ACKSSuTFu	—	ACSSuT	AK	SSu
		1974	208 (10)	ACKSSuTFu (3)	—	ACSSuT	AK	SSu
				ACKSSuTFu (2)	—	ACSSuT	AK	
				ACSSuTFu (5)	—	ACSSuT		SSu
			24 (2)	ACKSSuTFu (1)	—	ACSSuT	A	SSu
				ACKSSuTFu (1)	—	ACSSuT		
		1975	208 (20)	ACKSSuTTmFu (5)	—	ACSSuTTm	A	SSu
				ACKSSuTTmFu (4)	—	ACSSuTTm	AK	SSu
				ACKSSuTFu (6)	—	ACSSuT	AK	SSu
				ACKSSuTFu (2)	—	ACSSuT		SSu
				ACKSSuTGFu (2)	—	ACSSuTG	AK	SSu
				ACSSuTFu (1)	—	ACSSuT		SSu
		1976	208 (2)	ACKSSuTFu (1)	—	ACSSuT	AK	SSu
				ACKSSuTTmFu (1)	—	ACSSuTTm	A	SSu
			Untypable (2)	ACKSSuTTmFu (1)	—	ACSSuTTm		SSu

Table 3 (*cont.*)

Country of origin	Number of cultures	Year of isolation	*Salmonella typhimurium*		Drug resistance transfer					
								By mobilization†		
			Phage type	R-type*	Directly			F_1me	Group 1	Group 2
Jordan	1	1974	208	ACKSSuT	—			ACSSuT	AK	SSu
Kuwait	2	1971	208	ACKSSuT	—			ACSSuT	A	SSu
		1975	208	ACKSSuT	A††		SSu	ACSSuT		.
Syria	1	1974	208	ACKSSuTNx	—			ACSSuT	AK	SSu
Turkey	3	1973	208	ACKSSuT	ACKSSuT			.		
			208	ACKSSuT	—			CT	AK	SSu
		1975	208	ACKSSuT	—			CT	A	SSu
Uncertain‡‡										
(i)	1	1972	208	ACKSSuT	CT	A§	SSu§	.	.	.
(ii)	1	1974	208	ACKSSuT	—			ACSSuT	A	SSu
Total	201									

Drug resistance symbols: G, gentamicin; Tm, trimethoprim; Fu, furazolidone; Nx, nalidixic acid.

Number of strains shown in parentheses. Unless otherwise stated, cultures were isolated in Britain.

* R-type = drug resistance spectrum.

† The *fi*+ group F_{II} transfer factor X mobilized group 1 and group 2 resistance determinants, and F_1me plasmids; the *fi*− group I_1 transfer factor Δ mobilized only the group 1 and group 2 plasmids. Class 2 resistance transfer systems were formed after mobilization with both X and Δ.

All F_1me plasmids coding for streptomycin resistance also conferred resistance to spectinomycin, whereas the independent group 2 SSu resistance determinants do not code for spectinomycin resistance (see text).

‡ Index case infected in Tehran, Iran.

§ Transferred in Class 2 relationship by autotransferring F_1me plasmid.

‖ This culture also carried a trimethoprim (Tm) resistance plasmid of undetermined compatibility, mobilized by X but not Δ.

¶ Isolated in Iran.

** Isolated in Israel.

†† *fi*+ group I_1 Class 1 R factor; SSu determinant in Class 2 association.

‡‡ (i) Between Pakistan and Turkey. (ii) Egypt or Syria.

Table 4. *Group $F_I$me plasmids in* Salmonella typhimurium *of mainly Middle Eastern origin*, 1969–76

		F_I*me* plasmids				
		Autotransferring		Non-autotransferring*		
Country of origin	Year of isolation	R-type	No.	R-type	No.	Total
Britain	1974–5	.	0	ACSSuT	13	13
India	1974	.	0	ACSSuT	1	1
	1975	.	0	CT	1	1
Iran	1970	ACKSSuT	2	ACSSuT	1	3
	1973	ACKSSuT	1	.	0	1
	1976	.	0	ACSSuT	1	1
	1969–74†	ACKSSuT	19	ACSSuT	53	72
		CT	12	CT	12	24
		T	12	T	4	16
		ACSSuT	1			1
Iraq	1974	.	0	ACSSuT	2	2
Israel‡	1971–4	.	0	ACSSuT	20	
	1975	.	0	ACSSuTTm	9	
				ACSSuT	9	
				ACSSuTG	2	
	1976	.	0	ACSSuTTm	3	
				ACSSuT	1	44
Jordan	1974	.	0	ACSSuT	1	1
Kuwait	1971	.	0	ACSSuT	1	1
	1975	.	0	ACSSuT	1	1
Syria	1974	.	0	ACSSuT	1	1
Turkey	1973	ACKSSuT	1	CT	1	2
	1975	.	0	CT	1	1
Uncertain§						
(i)	1972	CT	1	.	0	1
(ii)	1974	.	0	ACSSuT	1	1
Totals			49		139	188
Percent			26·1		73·9	

Unless otherwise stated, F_I*me* plasmids were identified in *S. typhimurium* isolated in Britain.

* Mobilizable with X transfer factor.

† *S. typhimurium* isolated in Iran.

‡ *S. typhimurium* isolated in Israel.

§ (i) Between Pakistan and Turkey.
(ii) Egypt or Syria.

covalently bonded to form single plasmids which transferred intact and remained transferable from their new hosts (Anderson, 1968*b*, 1969; Anderson & Threlfall, 1970, 1974).

Transfer by mobilization

The transfer factors routinely used in the triparental cross for determinant mobilization are Δ, an *fi*$^-$ group I_1 factor (Anderson & Lewis, 1965*b*; Grindley

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Table 5. *Resistances coded for by 188 $F_I$me plasmids identified in* S. typhimurium *of mainly Middle Eastern origin*

Autotransferring			Non-autotransferring		
R-type	No.	Per cent	R-type	No.	Per cent
ACKSSuT	23	46·9	ACSSuT	106	76·3
CT	13	26·5	CT	15	10·8
T	12	24·5	ACSSuTTm	12	8·6
ACSSuT	1	2·0	T	4	2·9
			ACSSuTG	2	1·4
Totals	49 (= 26·1 % of total)			139 (= 73·9 % of total)	

et al. 1972) and X, an *fi*$^+$ F_{II} factor (Anderson, 1968*b*; Anderson & Lewis, 1965*b*; Anderson, Pitton & Mayhew, 1968; Anderson & Threlfall, 1974). Most resistance determinants tested have been mobilizable with either or both of these factors.

The non-autotransferring F_I*me* plasmids were mobilizable with the X transfer factor at a frequency of about 10^{-4} in triparental crosses, but mobilization was not detected with Δ. It has long been known that non-transferring resistance plasmids may show specificity of mobilization potentiality towards different transfer factors (Anderson & Lewis, 1965*b*; Anderson, 1968*b*), so that mobilization of the F_I*me* plasmids by X but not by Δ came as no surprise. A group F_{IV} R factor mobilized F_I*me* plasmids at a frequency similar to that of X.

The predominant F_I*me* resistance linkage group mobilized by X in 139 cultures of Middle Eastern origin was ACSSuT (106) (see Tables 3, 4 and 5), but the following were also mobilized: CT (15); ACSSuTTm (12); T (4); and ACSSuTG (2). All the cultures carrying the ACSSuTTm and ACSSuTG linkage groups came from Israel.

Tables 4 and 5 indicate that in 23 of 49 cases, cultures of R-type ACKSSuT, and carrying autotransferring F_I*me* plasmids, transferred the complete ACKSSuT linkage group. Thirteen of the remainder transferred CT; 12, T; and 1 ACSSuT. By contrast, mobilization of non-autotransferring F_I*me* plasmids by the X transfer factor yielded, with relatively few exceptions (see above), final recipients carrying only ACSSuT. The mobilized plasmid retained its F_I*me* character; no recombination had occurred between it and the X transfer factor. In other words the mobilization had yielded Class 2 resistance transfer systems, in which the resistance determinant and the transfer factor remained as independent plasmids. The same was true of mobilization by X of the ACSSuTTm and ACSSuTG resistance plasmids from strains isolated in Israel.

Mobilization of the prototype non-autotransferring F_I*me* plasmid NTP101 (ACSSuT), identified in the Burns Unit strain 14M6407, was also effected with group H_1 and H_2 R factors at a frequency of about 10^{-6}. The transferring complex so formed belonged to Class 2 in the case of H_1 factors, but apparently to Class 1 with H_2 factors. The resistance transfer systems formed by mobilization of F_I*me* plasmids with H_2 factors were displaced completely by introduction of a further H_2 factor in compatibility tests. By contrast, autotransferring F_I*me* resistance

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plasmids displaced the F_I*me* component only of the F_I*me*-H_2 recombinant: the H_2 component was unaffected. This phenomenon is under further investigation.

When strains carrying an F_I*me* plasmid and an H_1 factor were crossed overnight with K12 at 28 °C, the H_1 factor was transferred at its usual frequency of about 5×10^{-1}, but the F_I*me* plasmid, which remained independent, at about 10^{-4}. However, similar crosses with strains carrying F_I*me*-H_2 recombinant factors resulted in transfer of the recombinant at the characteristic transfer frequency of H_2 factors, about 10^{-1}.

The frequency of mobilization of F_I*me* plasmids was much lower with group H factors (10^{-6}) than with the F_{II} factor X (10^{-4}). No mobilization of F_I*me* plasmids was detected with autotransferring resistance plasmids of compatibility groups I_1, I_2, B, P, W, N and *com* 7.

Phage propagation

Strains carrying autotransferring F_I*me* plasmids supported the growth of phage $\mu 2$ or fd, which indicated that they formed F fimbriae. The strains carrying non-autotransferring F_I*me* plasmids, however, did not support multiplication of these phages, which indicated the absence of F fimbriae from such strains.

The efficiency of plating (EOP) of the 'female-specific' phage $\phi 2$ (Cuzin, 1965) was studied on K12 carrying the F factor, an autotransferring F_I*me* factor, a non-autotransferring F_I*me* factor, and on K12F$^-$. The EOP of phage $\phi 2$ on the F$^+$ strain was 10^{-2} of that on K12F$^-$. The F_I*me* plasmids inhibited plaque formation by $\phi 2$ to a slightly lesser degree, which was similar with the autotransferring and non-autotransferring plasmids. The plaques were reduced in size to the greatest extent by K12F$^+$.

Mobilization of K

After many attempts K was mobilized by X in the reference strain 14M6407. However, when this occurred it was the result of recombination between K and the ACSSuT linkage group in 14M6407, to form a non-autotransferring ACKSSuT linkage group, which was mobilized by X in a Class 2 relationship.

Mobilization of K was also effected by autotransferring F_I*me* resistance plasmids that lacked the K marker, in nine strains from which the original F_I*me* plasmid had been displaced by F-*lac*. The mobilization frequency was low, 10^{-7} to 10^{-8} in overnight triparental crosses. Subsequent crosses showed that recombination had occurred between the K resistance determinant and the mobilizing F_I*me* plasmid. The recombinant was indistinguishable from the wild autotransferring F_I*me* plasmids that possessed the K marker.

K was also mobilized at a similar frequency by autotransferring resistance plasmids belonging to compatibility groups H_1, H_2 and *com* 7. In each case a Class 1 resistance plasmid was formed, belonging to the compatibility group of the mobilizing plasmid.

Compatibility properties of F_I plasmids

The F_I*me* plasmids were compatible with plasmids of groups F_{II}, F_{IV}, F_V, B, H_1, H_2, I_1, I_2, N, P, W, *com* 7, *com* 9, of group G (Anderson, Threlfall, Frost & Carr, 1975) and four undesignated groups identified in this laboratory.

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Since F_I*me* plasmids were incompatible with the F factor in the extrachromosomal or integrated state, it was at first assumed that they were simply further representatives of the F_I group, first designated by Hedges & Datta (1972). Indeed, the ACKSSuT R factor TP160, isolated from the Algerian strain of *S. typhi* type 44, was used in the Enteric Reference Laboratory for some time as an F_I reference plasmid, as was another F-like *S. typhi* R factor isolated in France (Chabbert & Gerbaud, 1974). The phage type of the French strain from which this R factor was isolated belonged to a newly identified type, E13, and the plasmid concerned, numbered R162, has phage-restricting properties that determine the type specificity of E13 (E. M. de Saxe and E. S. Anderson, unpublished work). However, its phage restriction is different from that of the R factor of *S. typhi* type 44, and from F_I*me* plasmids in general. Moreover, its compatibility properties differ from those of the F_I*me* group, as will be seen.

Further exploration of the compatibility properties of the F_I plasmids revealed differences between individual members that enabled the group to be subdivided. This subdivision was effected by exploiting the compatibility properties of F_I plasmids with plasmids of other groups, specifically MP10, a single-copy plasmid, apparently non-autotransferring, carried by many strains of *S. typhimurium* and defined in some detail by Anderson & Smith (1972*a*), Smith, Humphreys *et al.* (1973) and Smith, Humphreys & Anderson (1974). This plasmid is *fi*+, but a recombinant between it and a K resistance determinant has undergone deletion of the *fi*+ region and is therefore *fi*− (Smith, Grindley, Grindley & Anderson, 1970); it is routinely used in compatibility studies because of the convenience of the K marker. Another recombinant, between MP10 and the A translocon, has produced a plasmid that is autotransferring at low frequency, an indication that MP10 is a defective transfer factor (Smith, Humphreys *et al.* 1973). The new A resistance plasmid, designated A*, has the same compatibility reactions as MP10 and its K recombinant. Another compatibility group used in these studies is H_1, of which all freshly isolated examples so far studied in our laboratory have been incompatible with the F factor in its extrachromosomal state. F is displaced by H_1 factors, but this is a unilateral property, in that the F factor cannot displace H_1 plasmids: it is always F that is lost (Smith, Grindley *et al.* 1973; Anderson, 1975*b*).

Table 6 shows the results of the compatibility experiments with the F, F_I (R162 and ColV), F_I*me*, MP10 and H_1 plasmids.

These compatibility reactions can be summarized as follows:

(1) The F factor is incompatible with itself, with F_I factors such as ColV and R162, F_I*me* autotransferring and non-autotransferring plasmids, and H_1, unilaterally, as explained above.

(2) The F_I factor R162 is incompatible with F, with other members of its own group such as ColV, and with F_I*me*. It is compatible with MP10 and H_1.

(3) F_I*me* is incompatible with F, the F_I group, its own group and MP10, but is compatible with H_1.

(4) MP10 is compatible with F, F_I and H_1. It is incompatible with F_I*me* and its own group.

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Table 6. *Compatibility properties of the F factor, F_I, $F_I me$, MP10 and H_1 group plasmids*

Group	F	F_I	$F_I me$		MP10		H_1
		R162 ColV	Autotransferring TP160; TP181, TP187	Non-autotransferring NTP101	$MP10_{36}$ A* K		TP123
F	INC	INC	INC		COM		INC (loss of F factor only)
F_I	INC	INC	INC		COM		COM
$F_I me$	INC	INC	INC		INC		COM
MP10	COM	COM	INC		INC		COM
H_1	INC (loss of F factor only)	COM	COM		COM		INC

cOM = compatible. INC = incompatible.

Group		Plasmids (other than F and ColV)
F_I	R162	from *S. typhi* type E13, France 1974.
$F_I me$	TP160	from *S. typhi* type 44, Algeria 1974.
$F_I me$	TP181	from *S. typhimurium* type 208, 15M3557, Iran 1975.
$F_I me$	TP187	from *S. wien*, England 1974.
$F_I me$	NTP101	from *S. typhimurium* type 208, 14M6407, England 1974.
H_1	TP123	from *S. typhi* Degraded Vi-strain, Mexico 1972.
MP10	$MP10_{36}$	*fi*$^+$ plasmid from *S. typhimurium* type 36.)
MP10	A*	recombinant between the A translocon of ASu and MP10.
MP10	K	recombinant between the K determinant of *S. typhimurium* type 29 (5M4136) and MP10.

(5) H_1 is unilaterally incompatible with F and is fully incompatible with other members of its own group. It is compatible with F_I. F_I*me* and MP10.

The 'F_I' plasmids can therefore be distinguished from each other by the following reactions:

(1) The F factor is unilaterally incompatible with group H_1.

(2) The F_I group is compatible with MP10 and H_1.

(3) The F_I*me* group is incompatible with MP10 but compatible with H_1.

Of these plasmids, F_I*me* constitutes by far the largest group, at least so far as the salmonellas are concerned.

The F_I factors in general, to which these three groups belong, cannot be distinguished genetically unless their compatibilities with H_1 and MP10 are tested.

In *S. wien* we have identified F_I*me* plasmids that can become compatible with the F factor in the integrated, that is, Hfr, state. These will be described later (McConnell, Leonardopoulos, Smith & Anderson, unpublished observations).

Resistance plasmids other than $F_I$me

The *S. typhimurium* strains in this series carried small (probably multicopy) non-autotransferring resistance plasmids. These were A or AK; and SSu (see Table 3). The A determinant, and an A^- segregant of AK, are incompatible with each other, and belong to compatibility group 1 of non-autotransferring plasmids (Smith *et al.* 1974; Smith, 1975), that is, they are homologues of NTP1, the A determinant of strain RT1 (Anderson & Lewis, 1965*b*). The SSu plasmid is incompatible with ASu (Anderson, Kelemen, Jones & Pitton, 1968). It thus belongs to compatibility group 2 of Smith (1975) and is therefore a homologue of NTP2, the SSu determinant of RT1. A few strains lacked A or AK; and others lacked SSu. When the F_I*me* plasmids were autotransferring, these small resistance determinants were transferred to new hosts in a Class 2 relationship with the main plasmid. When the F_I*me* plasmids were non-transferring, the small determinants transferred in a Class 2 relationship with the mobilizing transfer factor used in triparental crosses.

One strain isolated in Britain from a patient infected in Iran in 1976 carried an independent determinant for trimethoprim resistance, which was mobilized by X but not by Δ. It is compatible with A or AK and SSu. Another strain, found in a patient infected in Kuwait, carried A in a Class 1 relationship with an *fi*$^+$ I_1 R factor. This factor mediated transfer of the SSu determinant of the host strain in a Class 2 relationship (see Table 3).

The frequencies of mobilization of F_I*me*, A or AK, SSu and the Tm plasmids by Δ and X are summarized in Table 7.

Ampicillin, streptomycin and furazolidone resistances

F_I*me* plasmids conferred penicillin (= ampicillin) resistance on their carrier strains, of which the MIC was about 250 μg/ml. By contrast, the ampicillin MIC of strains carrying the A or AK determinant was about 2000 μg/ml. The lower ampicillin resistance conferred by the F_I*me* plasmid may result from the presence of only one copy of the plasmid, while the high ampicillin resistance of the A or

Table 7. *Mobilization frequency of $F_I me$, A or AK, SSu and Tm plasmids by Δ and X transfer factors from original* S. typhimurium *strains into K12*

	Transfer factors	
Plasmids	Δ	X
$F_I me$	0	5×10^{-4}
A or AK	10^{-4}	10^{-6}
SSu	3×10^{-5}	4×10^{-5}*
Tm	0	10^{-4}

* Once introduced into K12, SSu is mobilized at a frequency about 100 times higher with Δ (*ca.* 10^{-4}) than with X (*ca.* 10^{-6}). A and AK do not show this change.

AK-carrying strains is the probable result of multiplicity – 10–20 copies – of the respective plasmid (Smith, Anderson & Clowes, 1970; Humphreys, Grindley & Anderson, 1972; Smith *et al.* 1974).

The A and AK determinants also conferred cephaloridine resistance on their host strains, the MIC of which was 32 μg/ml. In contrast, the A region of $F_I me$ plasmids isolated from *S. typhimurium*, did not appear to code for such resistance. However, $F_I me$ plasmids from some of the *S. wein* strains studied, do code for cephaloridine resistance. NTP1, the standard A determinant (Anderson & Lewis, 1965*b*; Smith *et al.* 1974) which is present in a multiplicity of 10–20 copies per cell (Humphreys *et al.* 1972) produces an ampicillin MIC of 2000 μg|ml in the host strains, and the cephaloridine MIC of such strains is 32 μg/ml, the same as strains carrying A or AK of Middle Eastern origin. When the A translocon of NTP1 recombines with the Δ transfer factor, the resultant A–Δ resistance plasmid (Anderson, Kelemen *et al.* 1968; Anderson, 1969), which is present as only one copy per cell (Humphreys *et al.* 1972), gives MICs of 250 and 4 μg/ml for ampicillin and cephaloridine respectively. Although the A region of $F_I me$ plasmids confers a degree of ampicillin resistance similar to that of A–Δ on host strains, no cephaloridine resistance was detectable in these strains. which showed the same cephaloridine MIC as the sensitive control, 2 μg/ml. Because of the low values of the respective MICs, these MIC determinations, as well as those of A-Δ, were confirmed in four independent parallel titrations. Since the $F_I me$ A region does not seem to code for cephaloridine resistance, the ampicillin resistance resulting from carriage of $F_I me$ plasmids may be caused by synthesis of a different enzyme from the TEM type of β-lactamase coded for by NTP1 and its homologues. This subject will be discussed in detail in a later paper.

All $F_I me$ plasmids carrying the S marker also conferred resistance to spectinomycin, an indication that the resistance was caused by an adenylylating enzyme (Benveniste & Davies, 1973). The streptomycin resistance coded for was low, the MIC of carrier strains being about 32 μg/ml. The small independent SSu resistance plasmids did not code for spectinomycin resistance, an indication that the inactivation of the drug was caused by a phosphorylating enzyme. They conferred much higher streptomycin resistance, MIC 250 μg/ml, on their carrier strains.

Furazolidone resistance, probably chromosomal in origin, was present in all Israeli strains.

Phage types and phage restriction in S. typhimurium

The phage type of the *S. typhimurium* strains investigated in this series was predominantly 208, as can be seen in Table 3. However, two other phage-typing reactions were encountered, which were probably related to that type: type 170; and one in which the organism was resistant to all the typing phages and was thus designated untypable. All type 208 cultures carried F_I*me* plasmids, while type 170 did not. Type 208 is resistant to all the typing phages of the revised typing scheme of Callow (1959), but is sensitive to ancillary phages used for extension of that typing scheme (Anderson, Ward, de Saxe & de Sa, 1977). Type 170, in contrast, shows a characteristic pattern of sensitivity to the phages of Callow (1959). F_I*me* plasmids exercise a characteristic phage-restriction pattern: when they are transferred directly or by mobilization into type 170, this restriction converts 170 into type 208. Conversely, loss of an F_I*me* plasmid by type 208 converts it into type 170. Such loss may occur spontaneously, or by displacement of the F_I*me* plasmid by the F factor, with which it is incompatible. Introduction of F_I*me* plasmids into *S. typhimurium* type 36, which is sensitive to all 30 of the routine *S. typhimurium* typing phages, restricts the sensitivity of the host strain to at least 22 of the phages. The pattern of phage lysis so produced is characteristic, but does not conform to that of a recognized type of the scheme of Callow (1959) or Anderson *et al.* (1977). It has been given the provisional designation non-conforming (= NC) 10.

The difference between type 208 and the untypable strains depends on the reaction to only one ancillary typing phage, to which type 208 is sensitive but the untypable strain resistant. The untypable cultures were found to carry a small-plaque temperate phage that rendered type 208 untypable by restricting sensitivity to the differentiating typing phage.

Further investigation of phage restriction in the Middle Eastern series revealed the following features:

(1) Type 170 carries a large-plaque temperate phage that converts types with wide phage sensitivity, such as 135, sensitive to 26 of 30 typing phages, into type 170, sensitive to only eight phages.

(2) Type 208 carries the large-plaque phage and an F_I*me* resistance plasmid.

(3) The untypable drug-resistant strains carry the large-plaque phage, an F_I*me* plasmid, and the small-plaque temperate phage that converts type 208 into the untypable strain.

Type 135 may have been the starting point of the series, since a few sensitive cultures of that type were isolated in Iran before the onset of the type 208 outbreak. The sequence of events could thus have been:

$$\text{type 135} \xrightarrow[\text{phage}]{\text{large-plaque}} \text{type 170} \xrightarrow[\text{plasmid}]{F_I me} \text{type 208} \xrightarrow[\text{phage}]{\text{small-plaque}} \text{Untypable strain}$$

It can therefore be assumed that the cultures giving the three reactions to the *S. typhimurium* typing phages belonged to a single strain which caused widespread infection in the Middle East during the period covered by the study, 1969–76.

Table 8. *Distribution of F_1me plasmids in* S. typhimurium *other than from the Middle East*

Country of origin	Year of isolation	*S. typhimurium*		F_1me plasmids					Other plasmids detected
				Autotransferring		Non-autotransferring			
		Phage type	R-type	R-type	No.	R-type	No.	Total	
Britain	1975	193 (8)	AKTFu	.	0	T	8	8	AK resistance determinant
Belgium	1974	208 (2)	ACKSSuT	.	0	CT	2	2	AK and SSu resistance determinants
Germany	1975	200 (2)	ACSSuT	CT	2	.	0	2	SSu resistance determinant and ColIb (transferable)
		Untypable (4)	ACKSSuT	CT	2	CT	2	4	AK and SSu resistance determinants
Canada	1974	208*	ACSSuT	ACSSuT	1	.	0	1	0
Kenya	1972	NC10 (4)	ACSSuT	ACSSuT	4	.	0	4	0
	1974	NC10 (9)	ACSSuT	ACSSuT	9	.	0	9	*fi*⁻ group I_1 Class 1 R factor, R-type AT
	1975	NC10 (2)	ACSSuT	ACSSuT	2	.	0	2	0
Liberia	1976	Untypable (5)	ACKSSuT (4)	ACSSuT	4	.	0	4	AK resistance determinant, *fi*⁻ group I_1 transfer factor
			ACKSSu (1)	ACSSu	1	.	0	1	
Total		37			25		12	37	
Per cent					67·8		32·2		

* Bovine origin.

Three strains of phage type 24 of *S. typhimurium* were also identified in this series. One, from Iran, showed the R-type KTFu: it carried an autotransferring F_I*me* plasmid coding for tetracycline resistance only. Israel provided two type 24 strains, both belonging to R-type ACKSSuTFu and carrying a non-transferring ACSSuT F_I*me* plasmid. However, one also carried A and SSu resistance determinants. Loss of the F_I*me* plasmid from these three strains yielded lines of increased sensitivity to the *S. typhimurium* typing phages, but showing a lytic pattern that did not conform to a recognized type. Apart from their plasmid content, no relation has so far been demonstrated between these strains and the type 208 series that form the main subject of this paper, although a relation must be suspected.

General distribution of $F_I$me plasmids

In S. typhimurium

Although the highest frequency of F_I*me*-carrying cultures of *S. typhimurium* occurred in the Middle East, predominantly in type 208 and untypable strains, similar plasmids were found in cultures of the same serotype isolated elsewhere, as Table 8 shows.

Only two cultures from Belgium and one from Canada in this series belonged to type 208, although the four untypable cultures from Germany are related to 208 in a way similar to that described for Middle Eastern untypable strains. The five Liberian untypable cultures do not appear to possess this relationship: they are still under investigation.

The strain of type 208 of bovine origin isolated in Canada is of some interest. It has already been established that the R factors found in man are the same as those in animals (Anderson, Humphreys & Willshaw, 1975). Although no human infections with *S. typhimurium* carrying F_I*me* plasmids have so far been encountered in Canada, therefore, the possibility of such infection evidently exists, as the isolation of the bovine culture shows.

Fifteen of the remaining 25 of the total of 37 strains shown in Table 8 belonged to type NC10 (see above); eight belonged to type 193; and two to type 200. Twenty-five of 37 cultures transferred their F_I*me* plasmids directly, in contrast to the Middle Eastern cultures, where non-autotransferability predominated. Moreover, kanamycin resistance, present in most of the Middle Eastern series, was absent from these strains.

The 15 Kenyan strains were isolated from fatal cases of meningitis in children. They all showed the NC10 restriction pattern with the *S. typhimurium* typing phages, the first occasion on which this pattern had been encountered in wild strains. These cultures yielded phage type 135 by spontaneous segregation, which occurred with high frequency. All of 10 type 135 segregant colonies examined carried the MP10 plasmid (Smith, Humphreys *et al.* 1973), which is incompatible with F_I*me* plasmids (see section on compatibility properties). This explains the high segregation rate of these NC10 strains. The type 135 segregants had lost the F_I*me* plasmid, indicating that the parent strain belonged to type 135 until it was infected with that plasmid.

Plasmids other than those of the F_I*me* group were found in a number of these

Table 9. *Distribution of F_1me plasmids in salmonellas other than* S. typhimurium, S. typhi, S. oranienburg, S. newport, S. heidelberg, S. wien

Salmonella serotype	Country of origin	Year of isolation	Phage type	R-type	F_1me plasmids: Autotransferring R-type	Autotransferring No.	Non-autotransferring R-type	Non-autotransferring No.	Total
S. typhi	Algeria	1974	44	ACKSSuT	ACKSSuT	1	.	0	1
S. oranienburg	Indonesia	1976	.	ACKSSuT	ACKSSuT	4	.	0	4
S. newport	Indonesia	1976	.	ACSSuT	ACSSuT	1	.	0	1
S. heidelberg	Rhodesia	1974	.	AKSSuT*	T	1	.	0	1
				ACKSSuT*	ACKSSuT	1	.	0	1
S. wien	Algeria	1970	.	ACKSSuT†	ACKSSuT	1	.	0	1
(see text)	France	1972	.	ACKSSuT	ACKSSuT	1	.	0	1
	Britain	1974	.	ACKSSuT	ACKSSuT	3	.	0	3
		1974	.	ACKSSu	.	0	ACK	1	1
		1974	.	ACKSSuTTmNx	.	0	ACKSSuT	1	1
	Austria	1974	.	ACKSSuT	ACKSSuT	1	.	0	1

* These cultures carried non-autotransferring A and SSu determinants and a KColIb R factor.

† All *S. wien* cultures carried an ASSu determinant, one culture carried an F-like transfer factor and another a ColIa plasmid.

strains, as Table 8 indicates. A substantial proportion were the small A or AK and SSu plasmids, but two strains from Germany also carried a ColIb transfer system, and the nine NC10 cultures isolated in Kenya in 1974 carried an *fi*⁻ group I_1 Class 1 R factor of R-type AT.

In other salmonellas

The remaining salmonellas in which F_I*me* plasmids have been identified are presented in Table 9.

Apart from *S. wien*, which is discussed separately below, there were eight cultures, in all of which the F_I*me* resistance factor was directly transferable. A strain of *S. typhi* from Algeria, four of *S. oranienburg* from Indonesia, and one of *S. heidelberg* from Rhodesia, transferred ACKSSuT linkage groups. The strain of *S. newport* transferred its complete resistance spectrum of ACSSuT, but one of *S. heidelberg* from Rhodesia, of R-type AKSSuT, transferred only T as an F_I*me* plasmid. The Rhodesian strains also carried mobilizable A and SSu determinants, and a KColIb R factor, which suggests that a clone of *S. heidelberg* was involved, despite the difference between the two cultures in the F_I*me* resistance transfer pattern.

The Algerian *S. typhi* strain was isolated from a sporadic case of typhoid fever in 1974 (Anderson, 1975*b*). Its F_I*me* resistance plasmid converts *S. typhi* phage type A, sensitive to all 96 of the Vi-typing phages, into type 44, which is sensitive only to phage 44. This is a property common to all F_I*me* plasmids tested.

Of the other salmonellas, sufficient is known only about *S. wien* to warrant further description. This serotype caused an outbreak of at least 329 cases in Algeria in 1969 (Mered *et al.* 1970) and outbreaks totalling at least 1302 cases in France between 1970 and 1972 (Le Minor, 1972). Most of the infections were in paediatric hospital units. There were sporadic cases of *S. wien* infection in Britain from 1973 onwards, and a hospital outbreak of at least 23 cases in 1974.

All the *S. wien* cultures isolated in these outbreaks were drug-resistant and carried F_I*me* plasmids, most of which were autotransferring. A detailed description of *S. wien* cultures will be published later (McConnell, *et al.*, unpublished work).

Le Minor (1972) postulated that the original French infections were imported from Algeria. We believe that the identity of the plasmids carried by the Algerian, French and British strains supports the hypothesis that only one strain of *S. wien* is involved, which may have originated in Algeria. It probably reached Britain from France.

DISCUSSION

The studies described above started with the investigation of non-autotransferring resistance plasmids in the strain of *S. typhimurium* that caused the outbreak of enteritis in the Burns Unit in Birmingham. Subsequent events directed our attention to strains of the same serotype which caused infection in most countries of the Middle East. Phage type 208, or types related to it, predominated, and most of the cultures exhibited remarkably uniform plasmid content: an

F_I*me* plasmid coding for R-type ACKSSuT (in the transferring members), or ACSSuT (in the non-transferring members); and the independent, small, probably multicopy A or AK, and SSu resistance determinants in most strains. There was a uniform pattern of mobilization, in a Class 2 relationship, of the non-transferring plasmids by Δ, X, F_{IV} and H_1 transfer systems, and possible formation of unstable Class 1 recombinants between non-autotransferring F_I*me* plasmids and H_2 transfer factors. There was also mobilization of an independent K determinant by recombination with autotransferring F_I*me* plasmids, and with transfer systems of groups H_1, H_2 and *com* 7, to form Class 1 plasmids.

These properties suggest that we are probably dealing with a clone of *S. typhimurium*, which, by some means as yet undetermined, has become distributed in the areas mentioned over a distance of at least 2500 miles (over 4000 miles if the Birmingham outbreak is included). Its complement of plasmids was probably acquired very early in its epidemic history.

The question of transferability of the F_I*me* plasmids presents an enigma. Molecular studies to be described later have shown that even the non-transferring members are of the large single-copy type, about 90×10^6 daltons; while those that transfer are larger, but by only a relatively narrow margin. There is high molecular homology between the transferring and non-transferring groups (Willshaw, Smith & Anderson, unpublished work). The non-transferring members have evidently undergone a small deletion, rendering them transfer defective.

Transfer deficiency of F_I*me* factors was found only in *S. typhimurium* and *S. wien*. Transfer occurred from all other salmonellas in which these factors were encountered: *S. typhi*, *S. oranienburg*, *S. newport*, and *S. heidelberg*. Although the number of cultures of each serotype except *S. typhimurium* and *S. wien* was small, the results suggest that a special situation was present in the *S. typhimurium* strains (and probably in *S. wien*, as will be described later (McConnell *et al.*, unpublished work)). The almost invariable presence of independent A or AK and SSu determinants in *S. typhimurium* supports that suspicion, although similar determinants were also found in *S. heidelberg* from Rhodesia. Finally, the fact that the overwhelming majority of *S. typhimurium* cultures belonged to phage type 208, or to types closely related to 208, as we have shown by the phage restriction studies, suggests once again that a widely distributed clone of *S. typhimurium* was involved. Variations in the resistance spectrum of the F_I*me* plasmid were observed not only in wild cultures, but also occurred by spontaneous segregation in the laboratory, so that such variation does not weaken the clonal hypothesis.

Compatibility studies of the F_I*me* group of plasmids revealed an unexpected plurality in the F_I group. Indeed it was only as the result of these studies that it was realized that F_I*me* was a separate group, to which both the autotransferring and non-autotransferring plasmids belonged. This group is distinct from the F factor, and from the F_I group as represented by resistance plasmid R162 and ColV. Molecular studies which support this subdivision of F_I plasmids will be described later (Willshaw *et al.*, unpublished work).

The mobilization of K in the *S. typhimurium* strains described here, by

recombination with autotransferring plasmids of several groups, is probably analogous to the mobilization of tetracycline resistance (T) by the Δ transfer factor in strain RT1 of type 29 of *S. typhimurium* (Anderson & Lewis, 1965*a*, *b*), while mobilization of non-autotransferring F_I*me* plasmids such as ACSSuT by X is to some extent analogous to that of the resistance determinants A and SSu by Δ or T-Δ. However, this analogy is less close, since A and SSu are small multicopy plasmids, while the F_I*me* group, such as ACSSuT, albeit non-transferring, are large single-copy plasmids (Willshaw *et al.*, unpublished work). The precise mechanism of mobilization of the small plasmids is unknown, but it may differ from that of the large plasmids: in the latter instance it may involve complementation by the X transfer factor of a transfer defect in the F_I*me* plasmid, which may otherwise be a complete transfer system. Since propagation experiments with F-specific phages establish that strains carrying autotransferring F_I*me* plasmids form F fimbriae, while those with non-autotransferring members do not, at least part of the defect is in fimbrial synthesis. Complementation by the X transfer factor could thus consist in supplying the F fimbriae needed for transfer of the F_I*me* factor. However, it is not immediately apparent how mobilization of F_I*me* plasmids by H_1 factors could involve the provision of F fimbriae, which are not formed by bacteria carrying H_1 factors. The complementation in this instance may depend on other features, and in this respect the fact that the mobilization frequency of F_I*me* plasmids by H_1 factors is lower than that by X by a factor of 10^{-2} may be significant.

The state of non-transferring K in the parent strains of *S. typhimurium* is not clear. It is manifestly independent of the F_I*me* plasmids. It may be chromosomal or plasmid in nature. Its very low frequency of mobilization by autotransferring plasmids of several compatibility groups supports the former view, on the basis of recombination initially being needed between such plasmids and the K chromosomal region, followed by 'recombining out' of the segment concerned, a process analogous to the formation of F-prime factors. However, K was spontaneously lost from the wild strain 14M6407 at a frequency of about 0·5 % in a freshly picked clone grown in nutrient broth for 8 h, which favours its being plasmid in nature.

The similarity between K mobilization by autotransferring plasmids in these *S. typhimurium* strains, and that of T by Δ in RT1, suggests that the K and T resistance determinants may subsist in similar states in their host strains. This hypothesis is supported by the fact that, once mobilized, the K-transfer factor recombinant is transferred at the frequency characteristic of the mobilizing plasmid: 10^{-2} in overnight crosses with F_I*me* at 37 °C, 5×10^{-1} with H_1 at 28 °C; 10^{-1} with H_2 at 28 °C; and 10^{-1} with *com* 7 at 37 °C. There is a close analogy with T-Δ, which was initially transferred from RT1 at a frequency of $< 10^{-6}$ after recombination had occurred between T and Δ; and subsequently transferred in overnight crosses at a frequency $> 10^{-1}$, the characteristic transfer frequency of Δ in such crosses.

We believe that the *S. typhimurium* cultures carrying F_I*me* plasmids probably constitute a clone. This hypothesis is reinforced by observations on cultures of

S. typhimurium, over 400 in number, recently received from Turkey. These cultures, which will be fully described later, were isolated from severe infections in a paediatric hospital over a period of three years, 1973–6. Most carry an $F_I me$ plasmid and A or AK and SSu determinants. Although a few cultures belong to type 208, the great majority belong to an untypable strain similar to that identified in the Middle Eastern cultures described in detail in this paper. The Turkish cultures therefore thus probably constitute a subclone of the *S. typhimurium* type 208 that predominates in the Middle East.

Clonal distribution of drug-resistant *S. typhimurium* over a wide geographical area is not a new finding. It was first observed with phage type 29 of *S. typhimurium*, which caused an extensive outbreak of infection in bovines and man in Britain during the period 1963–70. This strain carried multiple drug resistance, the transfer of which was mediated by the Δ factor (Anderson & Lewis, 1965*a*, *b*; Anderson, 1968*a*, *b*). Extensive distribution of a clone has also been observed with phage type 193 of *S. typhimurium* in the South American sub-continent (Anderson, Threlfall, Carr & Frost, 1974), and with type 194 in Northern Europe (Anderson, Threlfall *et al.* 1975). The South American strains carried distinctive fi^+ group N plasmids which did not endow their carrier strains with sensitivity to the IKe phage. The Northern European strain of type 194 carried a T resistance determinant and a Δ-like transfer factor in a Class 2 relationship. These observations support the hypothesis that the strains were also clonal in nature (E. S. Anderson, J. A. Frost, J. M. Carr and E. J. Threlfall, unpublished work).

Studies such as those described in this article establish that it is no longer sufficient, in the investigation of infections with the enterobacteria, to identify, for example, the serotype of a salmonella. Characterization should be rendered more precise by phage-typing the organisms when possible, and distinctive biochemical markers should also be sought. Naturally, the strains should be routinely tested for drug resistance. However, the simple identification of resistance markers is inadequate. The plasmid(s) carrying them require exploration. Because of the enormous dispersal of resistance and other plasmids, epidemiological studies may now need to be carried to the genetic and even molecular level, although there are few laboratories in which molecular epidemiology can be followed to a satisfactory conclusion.

The increasingly frequent demonstration of the clonal distribution of plasmid-bearing strains of enteric pathogens over extensive land masses, of which we have given an example here, and of which the huge chloramphenicol-resistant Mexican typhoid outbreak in 1972–3, and the even larger and lethal Central American epidemic of drug-resistant Shiga dysentery from 1968 onwards, were striking examples, suggests that plasmids may confer properties other than those of drug resistance, enterotoxigenicity and haemolysin or surface antigen synthesis on their carrier strains. They may contribute, in ways as yet unidentified, to communicability and virulence. These are facets that demand further study.

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