Epidemiol. Infect. (1993), **110**, 459–468

Occurrence of F_I*me* plasmids in multiply antimicrobial-resistant *Escherichia coli* isolated from urinary tract infection

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(*Accepted 24 January 1993*)

SUMMARY

Plasmids belonging to the F_I*me* incompatibility group were found in seven different serogroups of multiply antimicrobial-resistant *Escherichia coli* isolated from patients with urinary tract infection (UTI) and living in south-east London. Although widespread in *Salmonella* spp., F_I*me* plasmids have only previously been described in *E. coli* in a strain of serogroup O15 K52 H1 responsible for an extensive and protracted outbreak of invasive community-acquired infection in south-east London in 1986. Our findings suggest either a wider background occurrence of F_I*me* plasmids in *E. coli* associated with UTI than previously reported or alternatively, the dissemination and subsequent molecular diversification of the F_I*me* plasmid associated with the epidemic strain of serogroup O15 K52 H1.

INTRODUCTION

In 1986 a year long outbreak of urinary tract infection (UTI) occurred in south-east London [1, 2]. The causative organism was a multiply drug-resistant strain of *Escherichia coli*, serogroup O15 K52 H1, which was associated with clinically-severe infections. The epidemic strain possessed a large self-conjugative plasmid of approximately 100 megadaltons (MDa) which belonged to the F_I*me* incompatibility (*Inc*) group [3] and coded for resistance to ampicillin, chloramphenicol, streptomycin, sulphonamides, tetracyclines and trimethoprim. This plasmid also coded for the production of the hydroxamate siderophore aerobactin, which is a known virulence factor for some enteric and urinary tract pathogens. The strain also carried a cryptic non-conjugative plasmid of 1·0 MDa which did not code for either drug resistance or aerobactin production.

F_I*me* plasmids were first identified in multiply drug-resistant *Salmonella typhimurium* of Middle Eastern origin [3, 4] and have subsequently been identified in epidemic strains of *S. wien* and *S. typhimurium* which have been commonly

associated with severe infections in paediatric hospitals in developing countries [5, 6], in sporadic drug-resistant isolates of *S. typhi* [7] and more recently, in strains of *S. typhimurium* isolated from turkeys and chickens in England [8]. However, before the outbreak of *E. coli* O15 K52 H1 in 1986, this plasmid group had not been described in *E. coli*.

The purpose of this study was to identify and characterize the plasmids responsible for mediating multiple drug resistance in serogroups of *E. coli* other than O 15 K52 H1, isolated from patients with UTI living in south-east London 4 years after the epidemic of *E. coli* O15 K52 H1 infection.

MATERIALS AND METHODS

Collection and identification of isolates

Organisms were identified as *E. coli* by their API-20E biochemical properties (API System, La Balme Les Grottes, Montalieu-Vercieu, France). Antibiotic susceptibility testing was performed by the Comparative disk diffusion method [9] on all *E. coli* isolated from urine specimens submitted for routine bacteriological investigation to the Microbiology laboratory at St Thomas's Hospital, London from August 1989 to May 1990. The specimens came from inpatients, outpatients attending clinics, and patients of general practitioners in south-east London, the area in which the previously-reported outbreak of infection with *E. coli* O15 K52 H1 had occurred.

Selection of multiply antimicrobial drug-resistant strains

For the purposes of this study, multiple antimicrobial resistance was defined as resistance to ampicillin (A), chloramphenicol (C), streptomycin (S), sulphonamides (Su), tetracyclines (T) and trimethoprim (Tm) (= R-type ACSSuTTm), this being both the R-type of the epidemic strain of *E. coli* O 15 K52 H1 and the resistances encoded by the F_1*me* plasmid carried by this strain. All isolates of *E. coli* with this resistance pattern were saved and maintained on Dorsets' Egg slopes stored at 18 °C. Since a small number of strains of the epidemic *E. coli* O15 K52 H1 also displayed resistance to kanamycin (K), susceptibility to this antimicrobial was also recorded, although not used as a criterion for selection. The designation and R-type of strains selected for further study are shown in Table 1.

Serogrouping

Isolates were O serogrouped at the Laboratory of Enteric Pathogens (LEP), Central Public Health Laboratory, Colindale according to the method of Ørskov and Ørskov [10].

Plasmid transfer

Multiply-resistant strains were tested for the ability to transfer resistance at both 28 and 37 °C to 14R519 (*E. coli* K12 F^- *lac*$^-$, nalidixic acid-resistant). The methods were those of Anderson and Threlfall [11] and counter-selection was exercised with nalidixic acid (40 mg/l). When no transfer of resistance was detected, mobilization of drug resistant determinants was attempted by standard

Table 1. *Serogroup, R-type and aerobactin production in* Escherichia coli *strains from UTI*

Strain	Serogroup	R-type*	Aerobactin production
E63905	O21	ACKSSuTTm	+
E63906	O21	ACSSuTTm	−
E63909	O2	ACSSuTTm	−
E63910	O9ab	ACSSuTTm	−
E63911	O21	ACKSSuTTm	+
E63921	O21	ACSSuTTm	−
E63925	O18ab	ACKSSuTTm	−
E63927	O8	ACKSSuTTm	−
E63929	O9a	ACSSuTTm	−
E63933	O2	ACSSuTTm	−
E63934	O75	ACSSuTTm	+
E63938	O71	ACSSuTTm	+
E63940	O8	ACSSuTTm	−
E63942	O75	ACSSuTTm	+
E63947	O21	ACSSuTTm	+
E63950	O21	ACKSSuTTm	+
E63952	O6	ACSSuTTm	+
E63953	O2	ACSSuTTm	+
E63955	O106	ACSSuTTm	+
E63956	O83	ACSSuTTm	−

* R-type, drug resistance pattern. Resistance symbols: A, ampicillin; C, chloramphenicol; K, kanamycin; S, streptomycin; Su, sulphonamides; T, tetracyclines; Tm, trimethoprim

Table 2. *Properties of* F_I*me plasmids in* Escherichia coli *from UTI*

No.	Host strain serogroup	R-type	Designation	F_I*me* plasmid		Aerobactin
				Resistances	MW (MDa)	
E63921	O21	ACSSuTTm	pDEP36	ACT	92	−
E63929	O9a	ACSSuTTm	pDEP37	ACSSuTTm	110	−
E63933	O2	ACSSuTTm	pDEP38	ACSSuTTm	110	−
E63934	O75	ACSSuTTm	pDEP39	ACTTm	100	−
E63938	O71	ACSSuTTm	pDEP40	ACSSuTTm	98	+
E63947	O21	ACSSuTTm	pDEP41	ACSSuTTm	110	−
E63952	O6	ACSSuTTm	pDEP42	ACTTm	90	−
E63955	O106	ACSSuTTm	pDEP43	ACT	90	+

Resistance symbols: see Table 1.
MW, molecular weight.

methods [11], using the conjugative plasmids X (42 MDa, *Inc*F_{II}) and Δ (60 MDa, *Inc*I_I).

Incompatibility grouping

Incompatibility between pairs of plasmids was tested for in accordance with the method of Grindley and colleagues [12]. Transconjugants containing the plasmids under investigation were prepared using 14R519 as the recipient and their antimicrobial susceptibility pattern determined using the methods of Anderson

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and Threlfall [11]. Plasmids were confirmed as belonging to the $F_I me$ group on the basis of their incompatibility with selected plasmids of defined groups [13] and also with the plasmids K-MP10 (*Inc*MP10), TP180 (*Inc*$F_I me$) and F-*lac* (*Inc*F_I) [4].

Preparation of plasmid DNA and gel electrophoresis

Plasmid DNA was extracted from wild-type strains and from *E. coli* K12 exconjugants, using a modification of the method of Kado and Liu [14] as described by Threlfall and colleagues [15]. Following electrophoresis at 100 V for 3 h on horizontal (BRL H5 Horizontal Gel Apparatus) Tris–borate agarose gels (0·7% w/v, Sigma Chemical Co. Ltd) and visualization under u.v. illumination after staining with ethidium bromide, molecular weights (MWs) were determined in relation to plasmids with MWs of 98, 42, 23·9 and 4·6 MDa. These plasmids were carried in *E. coli* K12, strain 39R861 [16]. The MWs, designations and resistance conferred by the $F_I me$ plasmids from those strains carrying this plasmid incompatibility group are shown in Table 2.

Restriction endonuclease fingerprinting and assessment of molecular relatedness

Plasmid DNA extracted as described above was further purified by filtration [17] and digested with *Eco*R I under conditions recommended by the manufacturers (Gibco BRL). After 4 h, digests were terminated and electrophoresed at 15 V for 18 h on 0·8% Tris acetate gels. After staining with ethidium bromide the results were recorded photographically and digitized. The resultant digest fingerprints were compared with those of pDEP10, an *Inc*$F_I me$ plasmid from *E. coli* O15 K52 H1, strain E44234, which was isolated during the 1986 outbreak.

The molecular relatedness of the *Inc*$F_I me$ plasmids was estimated on the basis of the number of fragments in common with pDEP10 after digest with *Eco*R I. and was numerically expressed by using the Dice coefficient of similarity [18] to compare the respective fragmentation patterns.

Detection of the phenotypic expression of aerobactin

Production of the hydroxamate siderophore aerobactin was determined by a cross-feeding bioassay [19]. M9 minimal salts medium [20] was supplemented with 0·5% casamino acids, 0·4% glucose, 0·005% thiamine and 0·002% tryptophan. and contained 160 μmol 2,2′-dipyridyl as an iron chelator. M9 agar plates were overlayered with M9 soft agar seeded with the aerobactin-requiring strain LG1522. The test strains were then spot inoculated onto the surface and incubated overnight. A positive result was indicated by a halo of growth of the indicator strain around the test organism. The wild-type strains and the transconjugants containing the $F_I me$ plasmids were examined by this method.

Detection of genes coding for aerobactin production and aerobactin–iron receptor function

This was performed on the wild-type and transconjugant strains by colony hybridization. Strains were grown on nylon filters (Amersham, Hybond-N) placed on nutrient agar. Bacterial colonies were lysed by treatment with alkali [21]. released DNA was fixed to the filter by exposure to short-wave u.v. light and the

filters were then gently washed and dried. Probe DNA was isolated from low-melting point agarose gels and labelled with ^{32}P by the method of Feinberg and Vogelstein [22]. The aerobactin biosynthesis probe was a 2 kilobase (kb) *Ava* I fragment prepared from pABN5 [23] and the aerobactin–iron receptor probe was a 2·3 kb *Pvu* II fragment prepared from pABN1 [24]. Filters were incubated overnight at 65 °C in the presence of denatured probe DNA and washed as previously described [25]. Strains showing hybridization with the probe DNA were identified following autoradiography.

RESULTS

Isolates

In the period of study 3821 *E. coli* were isolated from urinary tract specimens received from 3528 patients. A total of 130 isolates were of R-type ACSSuTTm. Twenty isolates with this R-type were selected randomly and characterized as above.

The results (Table 1) show that the 20 selected isolates were comprised of strains belonging to 11 serogroups, with serogroups O21 (6 strains), O2 (3 strains), O8 (2 strains) and O75 (2 strains) predominating. In addition to being resistant to A, C, S, Su, T and Tm, 5 strains were also resistant to kanamycin.

Occurrence and restriction endonuclease fingerprinting of F_1me *plasmids*

Eight of the 20 strains examined (40%) possessed conjugative F_1me plasmids. These strains belonged to seven different O serogroups (Table 2). The remaining 12 strains either contained conjugative plasmids which belonged to incompatibility groups other than F_1me (5 strains) or contained high MW plasmids which could not be mobilized by X or Δ (7 strains).

The 8 F_1me plasmids displayed 3 phenotypically different antimicrobial resistance patterns (ACSSuTTm, 4 plasmids; ACTTm, 2; ACT, 2). However, when cleaved with *Eco*R I, each F_1me plasmid exhibited a different restriction pattern (Fig. 1*a*, *b*). Furthermore, none of the patterns identified corresponded exactly with that of pDEP10, the prototype F_1me plasmid from the epidemic strain of *E. coli* O15 K52 H1. The plasmids with *Eco*R I fingerprints which most resembled those of pDEP10 were pDEP37, pDEP38 and pDEP40. These plasmids, isolated from strains of serogroups O9a, O2 and O71 all coded for ACSSuTTm. They were closely related to each other, with 14 of 15 restriction fragments in common with each other (pDEP37 and pDEP38) and 11 out of 14 for these plasmids and pDEP40, and also to pDEP10, with 12 bands in common for pDEP37 and pDEP38 and 10 bands for pDEP40. In contrast, *Eco*R I cleavage of the pDEP39 (coding for ACTTm) and pDEP36 (ACT) produced only 5 and 6 fragments respectively, of which only 3 were in common with those of the *Eco*R I digest of pDEP10. The 3 remaining plasmids had between 4 and 9 bands in common with each other and with pDEP10. All eight plasmids, including pDEP10 had in common a fragment of 1·42 kilobases (kb).

The Dice coefficients of similarity of these F_1me plasmids ranged from 93% between pDEP37 and pDEP38, to only 33% between pDEP39 and pDEP10, and

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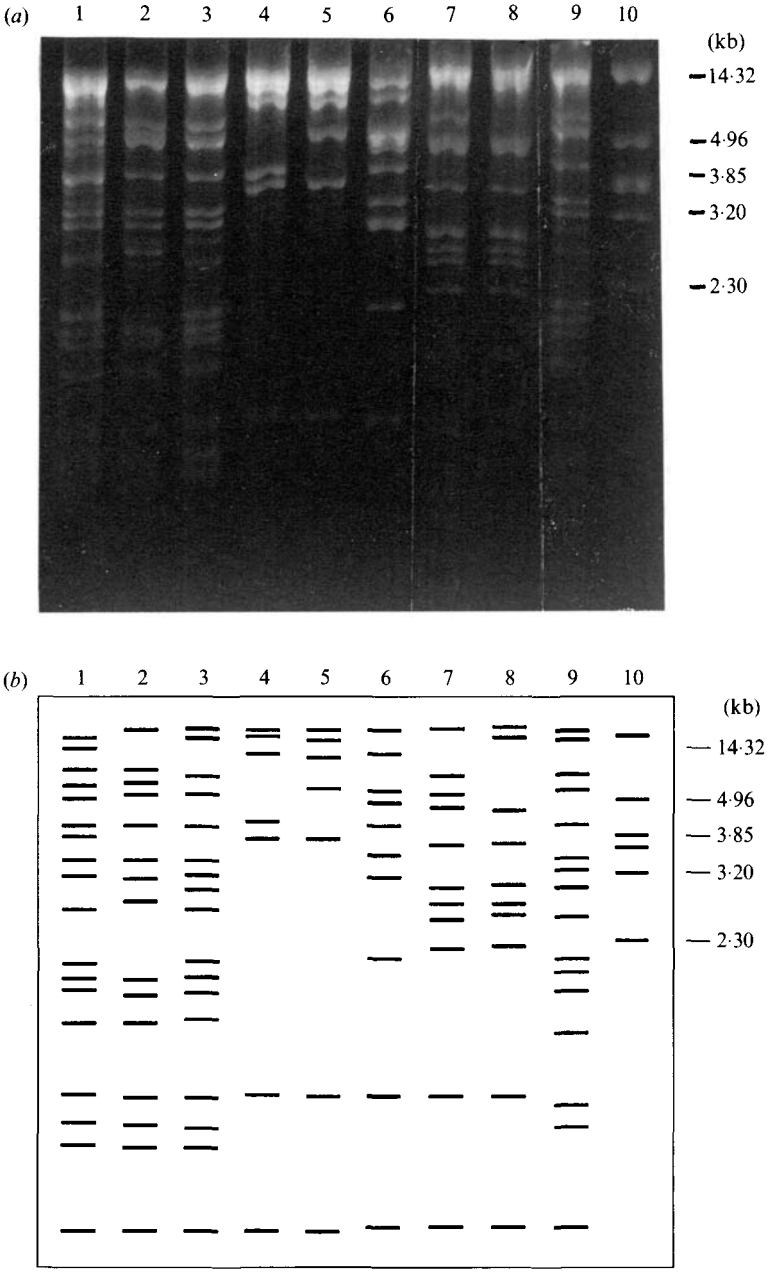

Fig. 1. *Eco*R I digest of F_1me plasmids from *Escherichia coli* isolated from UTI. (*a*) Track 1, pDEP10; 2, pDEP37; 3, pDEP38; 4, pDEP39; 5, pDEP36; 6, pDEP41; 7, pDEP42; 8, pDEP43; 9, pDEP40; 10, λ. (*b*) Digitization of above restriction enzyme fragmentation patterns.

pDEP43 and pDEP10 (Table 3). The three plasmids with the highest degrees of similarity with pDEP10 were pDEP37 (80%), pDEP38 (80%) and pDEP40 (70%).

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Table 3. *Dice coefficients of similarity between* F_I*me plasmids in* Escherichia coli *from UTI, expressed as a percentage*

Plasmid (pDEP)	40	43	42	41	36	39	38	37	10
10	70	33	40	47	27	33	80	80	100
37	79	36	43	50	29	21	93	100	
38	71	36	50	50	21	21	100		
39	50	75	75	50	75	100			
36	50	83	67	83	100				
41	56	56	44	100					
42	46	69	100						
43	40	100							
40	100								

Aerobactin production

Ten of the 20 strains examined produced aerobactin (Table 1), including 5 of the strains containing F_I*me* plasmids. These F_I*me*-carrying, aerobactin-producing strains were E63934, E63938, E63947, E63952 and E63955 but in only two of these strains (E63938, E63955) was aerobactin production found to be mediated by F_I*me* plasmids.

All wild-type strains and transconjugants found to be aerobactin producers by the cross-feeding bioassay also showed positive colony hybridization with the aerobactin biosynthesis and the aerobactin–iron receptor gene probes (data not shown). In contrast strains which were found not to produce aerobactin did not give positive colony hybridization results with the two probes.

DISCUSSION

Although identified in a range of salmonella serotypes [26], prior to this investigation F_I*me* plasmids coding for multiple antimicrobial drug resistance had to our knowledge only once been described in *E. coli*. The strain involved belonged to serogroup O 15 K52 H1 and was associated with severe community-acquired urinary tract infection in south-east London in 1986 [1, 2]. The F_I*me* plasmid from this strain, in addition to coding for resistance to A, C, S, Su, T and Tm, also coded for the production of the siderophore aerobactin. The outbreak lasted for about a year and the source of the causative organism, despite extensive epidemiological investigations, was not identified.

The results described here have important epidemiological implications. They demonstrate that F_I*me* plasmids are more widely distributed in *E. coli* than previously reported and in particular, that this class of plasmid may be associated with UTI. The possibility that there may have been dissemination of the F_I*me* plasmid from the epidemic strain of *E. coli* O 15 K52 H1 to the other serogroups of *E. coli* isolated from UTI was considered. However, with the possible exceptions of pDEP37, pDEP38 and pDEP40, such dissemination was thought to be unlikely. F_I*me* plasmids previously identified in different serotypes [27] and phage types [28] of salmonellas have been shown to have high DNA homology. Although genetic instability resulting in the loss of antimicrobial drug resistance has been

reported with F_I*me* plasmids in *S. typhimurium* phage type 208 [4], in *S. johannesburg* [29], in *S. wien* [30] and more recently, in untypable strains of *S. typhimurium* isolated in England [8], the coefficients of similarity between related plasmids remained in excess of 60% [8]. In this study, with the exception of pDEP37, pDEP38 and pDEP40, the coefficients of similarity between pDEP10 and the remaining F_I*me* plasmids were less than 50%, which is considerably less than has been observed from evolutionary divergence within a single F_I*me* plasmid type. Furthermore, the plasmids were also phenotypically heterogenous in respect of the antimicrobial resistance conferred and the production of aerobactin.

For the plasmids pDEP37, pDEP38 and pDEP40, a closer relationship with pDEP10 was observed. Although pDEP37 and pDEP38 did not code for the production of aerobactin, their drug resistances were identical with those of pDEP10 (ACSSuTTm) and their *Eco*R I fingerprints were very similar, with an 80% coefficient of similarity. In contrast pDEP40, with a 70% coefficient of similarity with pDEP10, did code for the production of aerobactin. All three plasmids were self-conjugative and the possibility of their evolutionary divergence from pDEP10 after its dissemination into strains of serogroups O21, O9a and O71 cannot be excluded.

F_I*me* plasmids have been associated with increased virulence in several different salmonella serotypes. Colonna and colleagues [27] examined 11 F_I*me* plasmids isolated from epidemic strains of *S. typhimurium* phage type 208 and *S. wien* and found all to be aerobactin producers, irrespective of serotype of origin. This hydroxamate iron scavenger may therefore be an important determinant of the increased pathogenicity associated with the strains and serotypes of salmonella known to carry F_I*me* plasmids. Furthermore aerobactin has been shown to be produced by a higher frequency in *E. coli* isolated from extraintestinal sites compared to faecal isolations from healthy individuals [25], and is considered to be an important virulence factor in UTI.

Despite previous observations, there is no reason to believe that aerobactin production should be a feature of all F_I*me* plasmids. The strains examined by Colonna and colleagues [27] were all clonal derivatives of either epidemic *S. wien* or epidemic *S. typhimurium* and were consequently very similar. Furthermore, it is known that the genes coding for aerobactin production and drug resistance can be spontaneously lost from F_I*me* plasmids [8]. The finding of IS*1* elements flanking the aerobactin iron-uptake system has led to the suggestion that a composite transposon may have facilitated the dissemination of the aerobactin system [27]. Absence of genes coding for the aerobactin system in F_I*me* plasmids would therefore not be unexpected and the finding that 2 of the 8 F_I*me* plasmids examined in this study encoded aerobactin production is indicative of the diverse origin of these plasmids. However it is noteworthy that 10 of the 20 strains of *E. coli* tested produced aerobactin and in 8 of these strains, the genes for aerobactin production were either located on plasmids belonging to incompatibility groups other than F_I*me* or were chromosomally located.

The pathogenicity of *E. coli* with aerobactin- and non-aerobactin-producing F_I*me* plasmids and isolated from UTI is currently under investigation and additional UTI strains are being examined to determine the distribution of multiresistant F_I*me* plasmids before the *E. coli* O15 K52 H1 epidemic.

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