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R-Plasmid Transfer In Vivo in the Absence of Antibiotic Selection Pressure

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The persistence of an O18 *Escherichia coli* strain resistant to tetracycline, streptomycin, and sulfonamide has been followed in the fecal flora of a single individual over a period of 9 months. The strain in question carrying an R plasmid was detectable from the beginning of the survey, but it was only after a 10-day period of tetracycline therapy that it reached an all but permanent dominance in the fecal flora. No transfer of the R plasmid carried by the strain to any other coliform could be detected for 202 days after the end of tetracycline treatment. At this point, however, an O88 *E. coli* carrying the same plasmid as the O18 strain appeared briefly as a predominant component of the flora. The two plasmids isolated from the O18 and the O88 *E. coli* strains have been characterized in molecular terms and found to be similar. This suggests that R-plasmid transfer between two *E. coli* strains occurred in an individual who was living a normal daily life and who was not receiving antibiotics.

Previous claims that R-plasmid transfer occurs in man under natural conditions have either been based on experiments in which appropriate marker strains have been fed to volunteers (4, 24) or have been inferred from epidemiological data (10, 18). In all cases, antibiotics have been present at or near the time of transfer, either given deliberately for experimental purposes or administered therapeutically.

In this study we have examined the fecal flora of a single individual for a period of over 9 months. Towards the beginning of this period, tetracycline was administered for a period of 10 days at therapeutic levels, but thereafter antibiotics were not knowingly present. The tetracycline therapy led to the establishment of an O18 *Escherichia coli* line carrying an R plasmid with the marker pattern Tc Sm Su. (Abbreviations of antibiotics are as follows: Tc, tetracycline; Su, sulfonamide; Sm, streptomycin; Ap, ampicillin; Cm, chloramphenicol.) This strain has been detected as a majority component of the fecal flora on two occasions (30 and 9 days) before the beginning of tetracycline treatment, and as a minority strain on four other occasions [36, 12, 2, and 1 day(s) before treatment]. After the end of tetracycline therapy, this strain remained prevalent in the same person for at least 7 months. Twice during this period (202 and 242 days), an O88 *E. coli* expressing a similar marker pattern also appeared briefly as a majority strain.

Molecular examination of the R plasmids carried by the O18 *E. coli* throughout the study and the plasmid present in the O88 *E. coli* on day 202 suggests that they form part of the same plasmid clone and that R plasmid transfer was therefore likely to have occurred between two distinct *E. coli* strains in the alimentary tract of a person not receiving antibiotics.

MATERIALS AND METHODS

Bacterial strains, plasmids, and bacteriophages. *E. coli* UB1139, a nalidixic acid-resistant derivative of *E. coli* K-12, was used as a host for the plasmids under examination. The characteristics of this strain together with those of UB281 (the strain used as recipient in the transduction experiments) and of UB1180 and UB1181 (the strains used for examination of the presence of the *fi* character) are listed in Table 1.

UB1180 was constructed from JC3272 (1) by transferring the F'*lac* factor JCFLO to it (1). UB1181 is a nalidixic acid-resistant derivative of the *E. coli* K-12 multiple auxotroph JN200, provided by M. Jones-Mortimer (Department of Genetics, University of Birmingham, England).

The plasmids used for compatibility tests together with their sources are listed in Table 2.

Bacteriophages MS2 (25), P1*kc* (16), and λC1857 *int-2* (29) were from laboratory stocks.

Media. Nutrient broth consisted of 1.6% (wt/vol) nutrient broth base (Difco) containing 0.5% (wt/vol) NaCl. Nutrient agar was prepared from heart infusion agar (Difco), with the addition, where necessary, of appropriate antibiotics. Lysed blood agar was prepared from blood agar base (Difco) supplemented with 5% (vol/vol) saponin-lysed oxalated horse blood. Minimal salts agar was prepared as described by Grinsted et al. (12).

TABLE 1. *Characteristics of standard E. coli K-12 strains used*

Strain	Genotype	Origin	Reference
UB1139	F⁻ *lac*⁺ *leu*⁻ *met*⁻ *thy*⁻ *nal*ʳ		(5)
UB1180	F'*lac*⁺ *his*⁻ *trp*⁻ *lys*⁻ *str*ʳ	F'*lac*⁺ derivative of JC3272	(1)
UB1181	F⁻*lac*⁻ *pro*⁻ *trp*⁻ *his*⁻ *arg*⁻ *ile*⁻ *met*⁻ *nal*ʳ	(See text)	
UB281	F⁻ *pro*⁻ *met*⁻ *nal*ʳ		(5)
JC6310	*trp*⁻ *his*⁻ *lys*⁻ *str*ʳ *recA56*	Dr. J. C. Clark	(5)

Drug resistance. Resistance to antibiotics was determined on lysed blood agar by conventional disk tests using "multodisks," unless otherwise stated. The multodisks (type U1, Oxoid Ltd., London) contained tetracycline (50 μg), streptomycin (25 μg), ampicillin (25 μg), sulphafurazole (500 μg), nalidixic acid (30 μg), nitrofurantoin (200 μg), colistin sulfate (10 μg), and chloramphenicol (50 μg).

In certain experiments the minimal inhibitory concentration (MIC) values for strains was determined by plating single colony-forming units on nutrient agar plates containing a range of concentrations of the antibiotic under test. The MIC was recorded as the lowest concentration that did not allow growth.

Conjugation experiments. Exponentially growing donor and recipient broth cultures (containing about 10^8 bacteria/ml) were mixed (0.5 ml of donor plus 1.5 ml of recipient) and incubated for 2 h at 37°C. Samples (0.1 ml) from these mating mixtures and from a series of 10-fold dilutions prepared from them were then plated on appropriate antibiotic agar. Individual colonies obtained on these plates were then purified by subculture on agar containing the same antibiotics.

In experiments to measure the frequency of transfer, samples were taken for plating on selective agar 30 min after mixing.

Transduction experiments. Phage P1*kc* was propagated on *E. coli* UB1139 carrying one of the plasmids under examination using the soft-agar method described by Adams (2). The transduction procedure used was essentially as described by Lennox (16).

Test for the presence of F-pili. The presence of F-pili was determined by following the multiplication of phage MS2 on *E. coli* UB1139 carrying one of the plasmids under examination in liquid medium by the method of Grindley and Anderson (11).

Test for fertility inhibition. The presence of the *fi* character was detected either by observing the inhibition of plaque formation by MS2 on *E. coli* K-12 F⁺ when a test plasmid was present in the host strain as well as the F factor (22) or by measuring the extent to which F'*lac* transfer was reduced by the presence of a test plasmid in the same donor cell (7).

Studies on incompatibility. Plasmids were allocated to an incompatibility group by their ability to coexist with plasmids of known compatibility characteristics. *E. coli* UB1139 containing the plasmid under test was mated for 2 h under standard conditions with *E. coli* JC6310 containing one of the standard plasmids listed in Table 2. The mating mixture was plated on minimal agar plates containing either spectinomycin (40 μg/ml) and kanamycin (30 μg/ml), or spectinomycin and carbenicillin (500 μg/ml), thus selecting for JC6310, and thereafter observing the presence and stability of the two plasmids in this strain by testing for the appropriate resistance markers in the two plasmids.

Colonies obtained in this way were purified by restreaking on agar containing the same pair of antibiotics. Isolates that had retained both the plasmids involved in the test were then passed through three successive overnight subcultures in 10 ml of nutrient broth in the absence of antibiotics, and a loopful of the final culture was then streaked on nutrient agar to obtain single colonies. Four clones arising in this way were tested from each mating and at least 100 colonies from each of these clones were scored for the persistence of the two plasmids by patching on agar containing each of the antibiotics used.

Isolation of plasmid DNA. Cleared lysates were prepared essentially as described by Thompson et al. (27). Deoxyribonucleic acid (DNA) was precipitated from these using polyethyleneglycol 6000 (10% wt/vol) and 0.5 M NaCl as described by Humphreys et al. (15). The precipitates were resuspended in SSC buffer (0.15 M NaCl plus 0.015 sodium citrate), extracted twice with chloroform, and then centrifuged in conventional ethidium bromide-CsCl gradients (23).

Radioactive plasmid DNA was prepared and measured as described previously (5, 12).

***Eco*RI endonucleolytic digestion of DNA.** The reaction was carried out in 0.1 M tris(hydroxymethyl)aminomethane buffer, pH 7.5, containing 0.01 M $MgSO_4$. Usually, 2 μl of *Eco*RI endonuclease preparation and either 20 μl of plasmid DNA or 2 μl of λ DNA were used, and the reaction mixture was made up to a total of 40 μl with buffer. Such preparations normally contained about 10 μg of DNA. Mixtures were incubated at 37°C for about 1 h, and the reaction was stopped by the addition of 10 μl of a solution of sucrose (40% wt/vol) and bromophenol blue (0.1% wt/vol) in 0.1 M ethylenediaminetetraacetate, pH 8.0. The effectiveness of the *Eco*RI endonuclease preparation was always cal-

TABLE 2. *Characteristics of plasmid-containing host strains used for compatibility tests*

Plasmid[a]	Source	Incompatibility group	Plasmid resistance pattern	Reference
R1-19K	This lab	FII	Kn	(5)
RP1	This lab	P	Ap Kn Tc	(12)
R144	N. Datta	Ia	Kn	(20)
R388 Cb	This lab	W	Ap Tp Su	(5)
R825	R. Hedges	N	Ap	(17)
R64G[a]	Y. Chabbert	C	Ap Cm Su	(28)

[a] All plasmids were carried in *E. coli* JC6310 (see reference 5 and Table 1).

ibrated against phage λ DNA to ensure complete digestion in a preliminary experiment.

The samples were analyzed in vertical agarose slab gels (0.7% wt/vol). The agarose was dissolved in tris(hydroxymethyl)aminomethane-borate buffer (21), containing ethidium bromide (0.5 μg/ml), and electrophoresis was carried out with the same buffer at a constant current of 60 mA for about 2 h.

The DNA bands were photographed by using illumination from a long-wavelength ultraviolet lamp (UVL 56, Blak-Ray Inc., Calif.).

The *Eco*RI endonuclease was prepared from *E. coli* JK29 essentially as described by Thomas and Davis (26). The strain JK29 was kindly provided by K. Murray (University of Edinburgh, Scotland). Lambda DNA was prepared from λCI857 *int-2* phage by phenol extraction. The particles were obtained by one-step growth in *E. coli* C600 growing in nutrient broth.

RESULTS

Sequence of strains. Table 3 shows the various O-antigen types and the antibiotic resistance patterns of the *E. coli* present as majority strains in the fecal flora of a single individual during the 303 days of the study. These strains were isolated as described previously (13). A therapeutic course of tetracycline (250 mg, twice daily) was administered for 10 days, but thereafter no antibiotic was given. By far the most persistent strain was *E. coli* O18 Tc Sm Su. An O18 *E. coli* strain with the same marker pattern was also present before the onset of tetracycline therapy (Table 3). On days −30 and −9, it was present as one of the majority strains in the flora, whereas on days −36, −12, −2, and −1 it could only be detected by plating fecal suspensions on agar containing tetracycline.

Towards the end of the survey, O18 *E. coli* strains were encountered with the marker pattern Sm Su, and on two occasions (days 202 and 242) a Tc Sm Su pattern was found in an O88 *E. coli*. The other resistant *E. coli* strains encountered in the fecal flora after the end of tetracycline therapy were an O86 Ap Sm Su Cm strain present as a majority component on days 12 to 14 and an O11 Tc Su strain which formed part of the majority flora at the end of the period of therapy and for 9 days afterwards. Before therapy began, an O81 Tc-resistant *E. coli* strain was predominant on day −6, and a small number of colonies of O17 Tc Ap Sm Su were detected, on selective agar only, on day −27.

Resistance patterns of the O18 and O88 *E. coli*. A more detailed examination of the resistance patterns of the O18 and O88 strains isolated in this study showed that all were cross-resistant to streptomycin and to spectinomycin. It seems probable therefore that the R plasmid carried in these strains specified the streptomycin-spectinomycin adenylylating enzyme (6).

E. coli O18 isolates that gave a resistance pattern of Sm Su with standard disks (see Materials and Methods) appeared on days 74, 87, and 130, and thereafter with increasing frequency until this phenotype seemed to displace the O18 Tc Sm Su strains from about day 223 onwards. A more careful comparison of the O18 strains expressing resistance to Tc Sm Su with those that were resistant to Sm and Su alone showed that the tetracycline resistance of the latter type was reduced, but not completely lost. Thus, strains with the pattern Tc Sm Su gave MIC values of 60 μg/ml against tetracycline when measured as single colony-forming units on tetracycline agar, whereas the strains that had the pattern Sm Su when tested with disks gave MIC values against tetracycline of about 10 μg/ml. A variant of this particular O18 *E. coli* that had lost its plasmid gave an MIC against tetracycline of about 1 μg/ml. These results suggest, therefore, either that a new plasmid became prevalent in the O18 strains in this survey around day 130 or that a mutation occurred in the plasmid specifying the high levels of tetracycline resistance to give rise to strains which were more susceptible to this antibiotic without being fully susceptible. This point has been investigated further and will be discussed elsewhere.

Tests for R plasmids and their incompatibility relationships. A total of 30 O18 *E. coli* strains from the series shown in Table 3 (those isolated on days −36, −30, −2, T3, T9, 14, 29, 39, 42, 68, 69, 72, 96, 97, 103, 122, 143, 147, 151, 153, 168, 175, 188, 228, 230, 235, 240, 246, 249, 257) were mated with *E. coli* UB1139 in a standard cross (see Materials and Methods) to see whether the resistance patterns were R plasmid mediated. One isolate of the O88 *E. coli*, isolated on day 202, was treated in the same way as were those of O11 (days 5, 9), O17 (day −27), O81 (day −6), and O86 (day 14). In all cases except the O81 *E. coli* strain, the resistance pattern of the donor was R factor mediated, since in all cases the full pattern could be transferred to UB1139 in a standard cross.

Transduction experiments with phage P1*kc* were then carried out to test whether the resistance markers Tc Sm Su were located on the same plasmid or not. All the antibiotic resistance markers of these plasmids were cotransducible.

Further investigation of the low-level tetracycline resistance phenotype (as, for example, in the O18 Sm Su isolates from days 147, 153, 168, 175, 228, 230, 235, 240, 246, 249, and 257) showed that UB 1139 Spc^R exconjugants (ob-

TABLE 3. *Sequence of E. coli strains in one patient before, during, and after treatment with tetracycline*

O type	Resistance pattern[a]	No. of isolates (out of 10 colonies from non-selective agar) exhibiting given reaction at stated no. of days[b]																							
	Days before therapy	−36		−33		−30		−27		−22		−20		−16		−12		−9		−6		−2		−1	
O1										2						10									
O8										1				2				1							
O10																				1					
O15																								3	
O17	Tc Ap Sm Su																								
O18		10		6		9		†		2															
O18	Tc Sm Su	†				1										†		1				†		†	
O27																				1					
O50																		1							
O57								7																	
O68								3										5		1					
O75																				5					
O81	Tc																			2					
O107/149										5		10		8				2				10		7	

	Days of therapy	T1		T2		T3		T5		T6		T7		T8		T9		T10	
O18	Tc Sm Su	†		†		10		10		10		10		10		10		8	
O11	Tc Su																	2	

	Days after therapy	1	2	3	4	5	6	7	8	9	11	12	13	14	17	18	20	21	22	27	29	31	32	33	35	37
O11	Tc Su	4	2			4	4	4	2	3																
O18	Tc Sm Su	6	8	10	9	6	5	6	6	3	2	†	†	3	7	7	2	10	6	8	4	6	5	10	4	1
O86	Ap Sm Su Cm											3	8	7												
O108											3															
NT[a]												2	2													

	Days after therapy	39	41	42	43	45	46	64	65	67	68	69	72	74	76	78	80	81	82	84	87	88	89	90	94	95
O9								5	1																	
O11																		1	1							
O15					3														1				1			
O17																		4								
O18	Tc Sm Su	10	6	6	6	5	6	†	9	10	3	4	1	5	6	10	10	1	3	†	9	10	9	10	4	10
O18	Sm Su													1							1					
O21								2	1																	
O24						1																				

	Days after therapy	96	97	100	103	105	112	115	117	118	122	123	125	129	130	132	137	138
O11																		1
O15		1	1															
O18	Tc Sm Su	9	9		2	3	10	8	†	†	5	10	6	†	1	6	10	†
O18	Sm Su														1			
O20									1	4								
O24									4	4								
O27									2									
O50									3	1				9	6			

	Days after therapy	140	143	147	150	151	153	168	171	175	183	186	188	189	193	197	200	202
O15			2										3	6	10	10		
O18	Tc Sm Su		5	†	†	†	†	†	†	†	2	1	4	4	†	†	10	2
O18	Sm Su			2		10	6	10	5	8	4							
O88	Tc Sm Su																	8

	Days after therapy	208	209	210	216	223	228	230	235	237	240	242	244	246	249	256	257
O9							1										
O15						8											
O18	Tc Sm Su	†	2	†	†	†											
O18	Sm Su		1				3	2	10	1	5	1		10	10	5	5
O21		1					2						1				
O24													3				
O40					3												
O75					1												
O86			5														
O88	Tc Sm Su											5					
O101						2											
O128					4												
NT					2						1	1	4				

[a] Abbreviations: Tc, tetracycline; Su, sulfonamide; Sm, streptomycin; Ap, ampicillin; Cm, chloramphenicol; NT, not typable with available sera.

[b] In instances where number is under 10, the remaining isolates proved not to be *E. coli*.

[c] Culture on selective agar showed presence of strain as a substantial minority component of flora.

tained when low-level resistance O18 strains were used as donors) also showed low-level tetracycline resistance. This variant of the Tet^R phenotype therefore seems to be plasmid coded.

A preliminary experiment carried out on one of the *E. coli* O18 Tc Sm Su strains from this series showed that it would propagate MS2 phage, and this suggested that the plasmid concerned was probably of the F incompatibility group. Accordingly, all the plasmids that had been transferred to strain UB1139 (see above) were tested for their incompatibility with R1-19K (see Materials and Methods). All the plasmids with either a Tc Sm Su or a Sm Su pattern showed a degree of incompatibility with R1-19K, but segregation of the two plasmids after growing for 6 h in broth free of antibiotics was very low; about 80 to 90% of the tested colonies retained both plasmids. For this reason, growth in antibiotic-free broth was extended to three successive overnight subcultures. After that period segregation had occurred in 50 to 95% of the tested colonies. However, individual clones carrying R1-19K and the test plasmid segregated at different rates after release of selection pressure even when they were isolated from the same mating experiment.

The plasmids conferring resistance to the O18 strains in this survey certainly therefore show a degree of incompatibility with R1-19K, but the results are not clear-cut and immediate. This partial incompatibility was also found with all the plasmids whether they specified high- or low-level tetracycline resistance. In contrast, plasmids showing full incompatibility with R1-19K normally segregated completely after growth for 6 h in the absence of selection pressure.

Examination of the Tc Sm Su plasmid from the O88 *E. coli* strain isolated on day 202 also showed this partial incompatibility with R1-19K, and this suggested that this plasmid from the O88 strain may be related to those found in the O18 series.

The *E. coli* O11, O17, O86 isolated on days 9, −27, and 14, respectively, were also tested for incompatibility with R1-19K. All were compatible, and therefore carried plasmids that were different from those in the O18 and O88 strains.

Representative plasmids from the O18 and from one of the O88 strains were also tested for incompatibility with standard plasmids of compatibility groups N, W, C, Ia, and P. No incompatibility was observed in any case.

Fertility inhibition and propagation of phage MS2. All the plasmids that had been transferred to strain UB1139 from the O18 series and from the single O88 strain tested were examined for their ability to propagate the F-pilus specific phage MS2 and also to inhibit the transfer of F'*lac* in a standard test for fertility inhibition (see Materials and Methods). All the plasmids examined in this series with the marker pattern Tc Sm Su, or Sm Su, whether derived from O18 or O88 *E. coli*, propagated MS2 to give an increase in phage number of at least 1,000-fold, inhibited the frequency of transfer of F'*lac* by at least a factor of 100, and also inhibited the lysis of these strains by phage MS2 when transferred into *E. coli* K-12 F^+. The combination of MS2 susceptibility, fi^+ character, and partial incompatibility with R1-19K suggests that the plasmid specifying resistance of Tc Sm Su in both the O18 and O88 *E. coli* isolates examined in this study were very similar in properties to a classical FII class plasmid, and the same was true of the plasmids specifying low-level tetracycline resistance.

R-plasmid isolation from *E. coli* O18 and O88. The R plasmids derived from the O18 strains isolated on days −36, −30, 14, 143, and 249 and from the O88 strain occurring on day 202 were isolated as covalently closed, circular DNA after transfer to UB1139. The isolates from days −36, −30, 14, and 143 showed a Tc Sm Su phenotype, whereas that from day 249 had a Sm Su pattern. Circular molecules were detected in all samples examined after isolation in CsCl-ethidium bromide centrifugation (23) and were sized by electron microscopy (5). Measurement of the contour lengths gave values close to 66×10^6 daltons in all cases. Thus, the plasmids from the O18 and O88 *E. coli* strains all carry plasmids of very similar size.

Cleavage of R plasmids with *Eco*RI restriction endonuclease. Plasmid DNA isolated from those strains examined by electron microscopy (see above) was digested with *Eco*RI restriction endonuclease, and the fragments were separated by electrophoresis in agarose gels. A digest of lambda DNA prepared with the same enzyme at the same time was used as a standard to obtain an approximate size of the fragments. Each plasmid examined gave nine bands ranging in molecular weight from about 15×10^6 to less than 10^6. Figure 1 shows the patterns obtained from the Tc Sm Su plasmids from the O18 strain isolated on day −36 with that found in the O88 *E. coli* isolated 248 days later. All nine bands in the two digests are in similar positions, and this argues that the two plasmids examined in this experiment are extremely similar. Cleavage studies on the plasmids obtained from the *E. coli* O18 strains isolated on days −30, 14, 143, and 249 also show nine bands in identical positions to those shown in Fig. 1.

Plasmid DNA isolated from strains O11, O86,

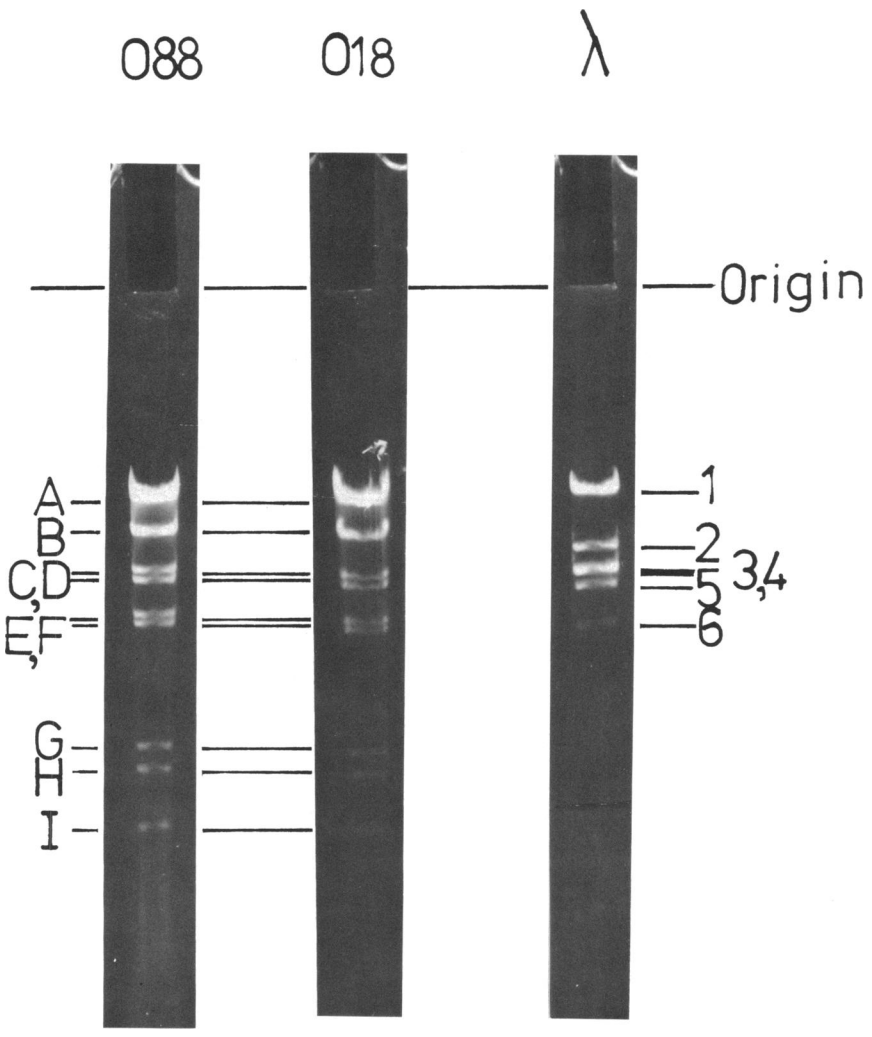

FIG. 1. *EcoRI restriction endonuclease hydrolysis patterns obtained from phage λ DNA and from the plasmids isolated from O18 and O88 E. coli studied in this survey. The six bands (numbers 1-6) obtained from phage λ DNA have molecular weights (× 10^6 d) of 13.7, 4.74, 3.74, 3.48, 3.02, and 2.13 (reference 26). Prolonged separation of the digest from the O18 and O88 plasmids shows that band B is double (data has shown). Electrophoresis was carried out in 0.7% (wt/vol) agarose gels as described. The photographs represent different sections of the same slab gel.*

and O17 (days 9, 14, −27, respectively) gave a completely different pattern when hydrolyzed with *Eco*RI when compared with the one obtained from the O18 and O88 Tc Sm Su plasmids.

DISCUSSION

The pattern of events in this survey of a single individual shows that antibiotic use can lead to the establishment of a resistant *E. coli* strain as a predominant complement of the fecal flora for long periods after the direct selective effect of the antibiotic has ceased (see also reference 14). A strain that had appeared sporadically before treatment became an all but permanent component of the flora afterwards, and it is difficult to escape the conclusion that the tetracycline treatment eliminated all susceptible competitors. The failure of the O17 Tc Ap Sm Su (day −27) and O81 Tc (day −6) strains to emerge at this time presumably means either that they were no longer present in the person's gut flora when therapy commenced or that the O18 strain had an additional selective advantage over the other two tetracycline-resistant *E. coli*. Thus, the sur-

vival of resistant *E. coli* in the gut of man seems to be subject to two main influences: the resistance of the strain in the presence of antibiotics and its competitive ability with respect to other bacteria (susceptible or resistant) once the pressure has been removed (14). Thus, antibiotic use is only one of the pressures that leads to the emergence of resistant strains as majority components of mixed populations, and there is no evidence that the carriage of an R plasmid is necessarily a disadvantage, as has been claimed by some (3, 8).

The similarity of marker phenotype, *fi* character, production of F-pili, partial incompatibility with R1-19, and the *Eco*RI endonucleolytic digestion patterns suggests that plasmid transfer may have occurred between the resident O18 *E. coli* followed in this survey and an O88 strain. The alternative possibility is that the serotype O18 strain was converted to O88. This seems improbable since the change would involve the loss of all rhamnose and the gain of mannose and 6-deoxytalose in the lipopolysaccharide, that is, a change from chemotype VII to chemotype XXVI (19). The possibility that the O88 Tc Sm Su plasmid might have appeared exogenously rather than by transfer is also improbable, since it is unlikely that the only Tc Sm Su plasmid to appear in the flora of the individual over a 9-month period should be identical in so many properties with that of the plasmid present throughout. Other studies on Tc Sm Su plasmids of FII compatibility group, isolated elsewhere, have never shown the same banding pattern with *Eco*RI endonuclease as the Tc Sm Su plasmid studied here in the *E. coli* O18 and O88 strains. Nor have any of these other plasmids ever shown partial incompatibility with R1-19K.

Perhaps the most surprising fact to emerge from this survey, and one of great importance for our understanding of the ecology of antibiotic resistance in *E. coli*, is that transfer of the plasmid in question to other *E. coli* could not be detected during a period of nearly 7 months after the strain became established. If transfer did occur the progeny did not survive well enough to become majority components of the fecal flora. So, although plasmid transfer seems to occur in the human gut, these observations, which were a survey of natural events, certainly did not suggest that the phenomenon occurred widely. Whether the process does occur more commonly, but the resulting strains are unable to survive well enough to become majority components of the flora, is unclear. Certainly, there was no evidence that any such transfer had occurred from examination of the samples plated on selective agar during this survey.

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