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Spread of R-Plasmids Among *Escherichia coli* Causing Urinary Tract Infections

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The incidence of multiple-antibiotic resistance among *Escherichia coli* isolated from urinary tract infections at Charing Cross Hospital, London, increased over the last 10 years, and its distribution was related to O-type. Among strains of the eight O-types most frequently causing such infections at this hospital, O4, O9, and O18 had a high incidence of multiple resistance (35, 22, and 19%, respectively); O2 and O6 had an intermediate incidence (14 and 11%, respectively); and O7, O1, and O75 had a low incidence (8, 6, and <3%, respectively). This nonrandom distribution appears to be a consequence of unequal plasmid recipient ability. After overnight mating with antibiotic-resistant donors, R-plasmid infection frequencies among antibiotic-sensitive urinary tract isolates differed by up to 10^5-fold, and such differences were correlated with the variation in the incidence of antibiotic resistance among the O-types. The inherent differences in the ability to achieve significant R-plasmid spread, which appear to be determined by the host, not the plasmid, may be compounded in some cases by the inhibition of potential mating partners by colicin production.

The distribution of antibiotic resistance (R) plasmids throughout *Escherichia coli* populations is not random. A study of *E. coli* isolated from human feces (9) has demonstrated that strains of O-antigen types O8, O9, and O101 occur at a far higher frequency among antibiotic-resistant isolates (whether shown to carry R-plasmids or not) than among isolates that are antibiotic susceptible. These O-types are also found to be prevalent among antibiotic-resistant strains isolated from calf feces. A further study has shown that a difference also exists between the O-types common among the antibiotic-susceptible and -resistant populations of *E. coli* causing urinary tract infections; O-types O4, O8, O9, O17, and O18 occur at a relatively high frequency in the R-plasmid-bearing group (16).

The majority of urinary tract infections are caused by the predominant O-type in the patient's own feces at the time of infection (7, 18), but the proportion of infections caused by antibiotic-resistant strains could be affected by such factors as relative colonization and survival ability in the urinary tract and chemotherapeutic practice. Among the various O-types, colonization and survival ability might be affected to differing degrees by the carriage of R-plasmids.

Whether R-plasmid distribution among urinary tract *E. coli* is indeed determined by such factors or reflects unequal competence of the strains to accept or disseminate plasmids is not known. This distribution is important as it dictates the effectiveness of hospital chemotherapeutic practice and because R-plasmid carriage may influence bacterial sensitivity to serum (17, 22, 24), a characteristic which appears to be important in urinary tract pathogenesis (6, 13, 15, 23).

In this study we report on the extent of R-plasmid spread observed after their introduction into populations of O-typed *E. coli* isolated from patients with urinary tract infection.

MATERIALS AND METHODS

Incidence of antibiotic resistance among isolates from urinary tract infections. The incidence of antibiotic resistance among the *E. coli* O-types used in this study was assessed by examining the records for all such strains isolated from patients attending the urinary infection clinics at Charing Cross Hospital during the years 1967 to 1979. The antimicrobial agents assessed routinely change with time, but during this period six were used consistently: ampicillin (Ap, 25 μg), colistin sulfate (10 μg), kanamycin (Km, 30 μg), nalidixic acid (Nal, 30 μg), nitrofurantoin (200 μg), and sulfafurazole (Su, 500 μg) tested as impregnated filter paper disks. The agents assessed routinely are, of course, chosen for clinical usefulness, not determination of plasmid-mediated resistance, which has so far been demonstrated for only three (ampicillin, kanamycin, and sulfafurazole) of the six used consistently.

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Of the isolates retained in our culture collection, 91 (37 resistant to ampicillin, kanamycin, and sulfonamide and 54 susceptible to these antibiotics) were further examined for susceptibility to cephaloridine (Cr, 15 μg), chloramphenicol (Cm, 10 μg), streptomycin (Sm, 10 μg), and tetracycline (Tc, 10 μg). Of the 54 strains susceptible to ampicillin, kanamycin, and sulfafurazole, 1 was found to be resistant to streptomycin, and five were resistant to tetracycline, whereas in the 37 resistant strains additional resistance was found to streptomycin (in 26), to tetracycline (in 19), to chloramphenicol (in 11), and to cephaloridine (in 11). This indicated that less than 14% of resistant strains were likely to have been overlooked in our initial survey and that resistance to the antibiotics ampicillin, kanamycin, and sulfafurazole could therefore be taken as an index of the relative frequency of R-plasmid-mediated antibiotic resistance among these O-types.

Bacteria used in mating experiments. All wild-type *E. coli* were isolated by culture of suprapubic aspirates of urine from patients attending the urinary tract infection clinics at Charing Cross Hospital between 1972 and 1979 and were identified by standard methods (3). Isolates were grouped with antisera prepared against *E. coli* serotypes O1, O2, O4, O6, O7, O9, O18, and O75, the O-type predominant in urinary tract infections treated at this (19) and other hospitals (7, 20). The sera were obtained from the Biological Reagents Section, Centers for Disease Control, Atlanta, Ga., and reacted with the homologous strains at titers of 1 in 1,280 or greater; heterologous reactions were less than 1:1,000.

To compare recipient ability, matings were performed between *E. coli* K-12 (Str^r or Nal^r) donor strains and rifampin (Rif)-resistant urinary tract isolates of different O-types which appeared to carry no R-plasmids. These were isolates susceptible to all of the following: ampicillin (10 μg), cephaloridine (15 μg), chloramphenicol (10 μg), kanamycin (30 μg), streptomycin (10 μg), sulfafurazole (500 μg), and tetracycline (10 μg). The *E. coli* K-12 donor strains carrying R-plasmids of defined *inc* character are listed in Table 1, as are the four multiple-resistant urinary tract isolates which were taken to represent R$^+$ donors currently prevalent in infections. These four strains had previously been shown to transfer all their resistances to *E. coli* K-12 M560, although it is not known whether all of the resistance determinants are, in each case, carried on one plasmid.

To compare donor ability, variants of Rif^r urinary tract isolates carrying the R-plasmid R1 were mated with M560, a Nal^r variant of the nonrestricting *E. coli* K-12 strain 803 described by Wood (25). Rif^r derivatives of strains were isolated as spontaneous mutants growing on Trypticase soy agar (TSA; BBL Microbiology Systems) containing 50 μg of rifampin per ml.

Mating. (i) Broth. An overnight Trypticase soy broth (TSB; BBL) culture of donor strain was diluted ca. 1:100 in the same medium and grown to midlogarithmic phase. It was then mixed with a stationary-phase TSB culture of recipient, giving each strain an initial concentration of ca. 5×10^7 viable cells per ml, and the mixture was incubated overnight without shaking. Initial and final viable populations of donor and recipient were counted on rifampin or nalidixic acid (50 μg/ml) TSA, and transconjugants were enumerated on rifampin/ampicillin (50 μg/20 μg per ml) or nalidixic acid/ampicillin (50 μg/20 μg per ml).

(ii) Solid medium. A 0.2-ml amount of logarithmic-phase donor culture (in TSB) was added to 0.1 ml of recipient overnight culture, and the mixture was spread on TSA. After overnight incubation, the mixture was removed, diluted in saline, and spread on selective medium.

Successful transfer by either method was confirmed by testing purified colonies for inheritance of other markers. The results obtained from conjugations were expressed as infection frequency, i.e., the proportion of the recipient population infected with the R-plasmid. This term is not synonymous with transfer frequency, as it reflects dissemination of the plasmid throughout the population, achieved by initial transfer from the donor and secondary transfer within the recipient population. It is used here as a relative index to compare the ability of various O-types to achieve significant R-plasmid spread. R-plasmid transfer is often more efficient on solid media than in broth (2, 5); therefore except for R1 and R46, which we knew to transfer sufficiently well in broth, plasmids were transferred on agar.

TABLE 1. *E. coli donor strains*

Strain[a]	R-plasmid[b]	Inc	Resistance
SFI 587 K-12 C600	R1	FII	Ap Cm Km Sm Su
SFI 463 K-12 C600	R46	N	Ap Sm Su Tc
W 274 K-12 C600	R69-2	M	Ap Km
W 269 K-12 C600	Rs-a	W	Cm Km Sm Su
W 279 K-12 C600	TP114	I2	Km
W 282 K-12 C600	RIP162-2	FI	Ap Tc
SPA 441 (O18)	Wild	Unknown	Ap Cm Km Su
SPA 481 (O4)	Wild	Unknown	Ap Cm Km Su Tc
SPA 5660 (O6)	Wild	Unknown	Ap Cm Sm Su Tc
SPA 1977 (O9)	Wild	Unknown	Ap Cm Su Tc

[a] *E. coli* K-12 C600 strains were obtained from the culture collections at the University of Würzburg (W) or Sandoz Forschungsinstitut (SFI). Wild-type *E. coli* were isolated by suprapubic aspiration (SPA) from urinary tract infections treated at this hospital; O-types are given in parentheses.

[b] The R-plasmids are described in reference 12, which quotes more detailed sources.

Colicinogeny. Colicin production was detected by the overlay method (14), using the colicin-sensitive indicator KH215, and assignment to group A (including colicins A, E, K, L, N, and X) or B (including colicins B, D, H, I, and V) was determined by action on the mutant indicators KH419 (*tolA*) and KH850 (*tonB*) (4, 11). Identification of individual colicins of group A was facilitated by the use of a wider range of mutant indicator strains as described previously (4). Responses were controlled by the use of the standard colicinogenic strains MI252 (ColE1$^+$) and KH932 (ColV$^+$). All of these strains are variants of *E. coli* K-12 and were obtained from the University of Kent, England, either through G. G. Meynell or K. G. Hardy.

For mating experiments designed to eliminate the effect of colicin production, colicin-insensitive variants of M560 and SFI 587 were isolated as colonies growing in inhibition zones produced by the relevant wild-type *E. coli* or standard colicinogenic strains. All colicin tests were performed on seed agar (BBL).

Stability of R-plasmids in urinary tract isolates. One R1$^+$ derivative of recipients belonging to each of the O-types O4, O18, O75, O1, and O7 was incubated under the same conditions employed for broth mating (i.e., overnight culture and subsequent subculture in TSB). Resulting cultures were spread on TSA containing either rifampin or rifampin/ampicillin to ascertain whether R-plasmid carriage was maintained.

Growth of R$^+$ progeny and R$^-$ parent strains. The 5 R1$^+$ progeny mentioned above and their R$^-$ parent isolates were grown separately in TSB after 1:10^4 dilution of overnight culture. Measurements of absorbance at 600 nm and colony counts made on TSA throughout the growth period allowed an assessment of the effects of R1 carriage on the growth rate of the isolates.

RESULTS

Distribution of antibiotic resistance among *E. coli* O-types. The O-types examined in this study, O1, O2, O4, O6, O7, O9, O18, and O75, are those most prevalent in urinary tract infections both at Charing Cross Hospital (19) and other hospitals (7, 20). For instance, in this hospital a recent study showed that these O-types were, respectively, responsible for 7, 2, 5, 10, 3, 2, 5, and 9%, i.e., a total of 43%, of infections (19). The results of the survey of resistance to ampicillin, kanamycin, and sulfafurazole among these O-types in the records at Charing Cross Hospital are shown in Table 2. The numbers are given separately for the years 1972 to 1979 (in which strains used in the present study were isolated) and for the previous 5 years. Although there was little difference between the two periods in resistance to ampicillin or sulfafurazole alone, resistance to two or more agents was twice as common in 1972 to 1979 (12.6%) as in 1967 to 1971 (5.7%), the increase being most apparent for types O4 and O18; the trend of increasing incidence of multiple resistance previously indicated for the years 1962 to 1969 (21) has clearly continued. The results agree well with those obtained earlier for both antibiotic resistance and proven R-plasmid carriage (16): O-types O4, O9, and O18 were resistant with high frequency, O1, O7, and O75 were resistant with low frequency, and O2 and O6 were resistant with intermediate frequency.

R-plasmid spread among urinary tract

TABLE 2. *Resistance to ampicillin, kanamycin, and sulfafurazole among E. coli isolated from urinary tract infections*[a]

O-type	1972 to 1979				1967 to 1971			
	No. of isolates	No. resistant to:			No. of isolates	No. resistant to:		
		Sulfafurazole only	Ampicillin only	Multiple[b] (%)		Sulfafurazole only	Ampicillin only	Multiple (%)
O4	23	3	0	8 (35)	13	0	0	2 (15)
O9	9	3	0	2 (22)	2	0	1	1
O18	32	9	1	6 (19)	14	3	1	1 (7)
O2	14	2	1	2 (14)	2	1	0	0
O6	44	3	3	5 (11)	30	6	1	1 (3)
O7	13	3	0	1 (8)	6	1	0	0
O1	36	4	0	2 (6)	14	3	0	0 (<7)
O75	36	5	0	0 (<3)	24	1	0	1 (4)
Total	207	32	5	26 (12.5)	105	15	3	6 (5.7)

[a] *E. coli* strains isolated from outpatients attending Charing Cross Hospital during the years 1972 to 1979, the period during which the strains studied in the present work were collected, compared with the previous 5 years.

[b] Number and percentage of strains resistant to at least two of the agents. Resistance to kanamycin was shown by five strains during 1972 to 1979 and by two strains during 1967 to 1971; in every case this was in conjunction with resistance to ampicillin, sulfafurazole, or both.

isolates. To assess the ease with which R-plasmids could spread among populations of the above O-types, antibiotic-susceptible isolates were mated in overnight culture with *E. coli* K-12 carrying the plasmid R1. Figure 1 shows the mean infection frequencies (final incidence of R-plasmid-bearing strains among the recipient population) achieved in 46 isolates belonging to the eight O-types. Large differences in infection frequency can be observed between the O-types, and these correspond to the variation in antibiotic resistance evident among urinary tract isolates. When the eight O-types are ranked from the highest to the lowest incidence of antibiotic resistance (table 2) and again from the highest to the lowest mean infection frequency (O4, O18, O9, O6, O75, O2, O7, O1), the two are significantly correlated (Spearman rank correlation coefficient, r_s, 0.786; $P < 0.05$). This is emphasized if the recipients are grouped into the O-types which are antibiotic resistant with low (O7, O1, and O75), intermediate (O2, O6), and high (O4, O18, O9) incidence. Although one third of the high-incidence isolates achieved an infection frequency higher than 10^{-4} (1% of the control *E. coli* K-12 M560 level), no strain of the low-incidence group attained this degree of R-plasmid spread. Further, a 10^{-6} infection frequency was not reached by 84% of the low-incidence isolates compared to only 10% of those belonging to the high-incidence group.

In several of the matings, very low infection

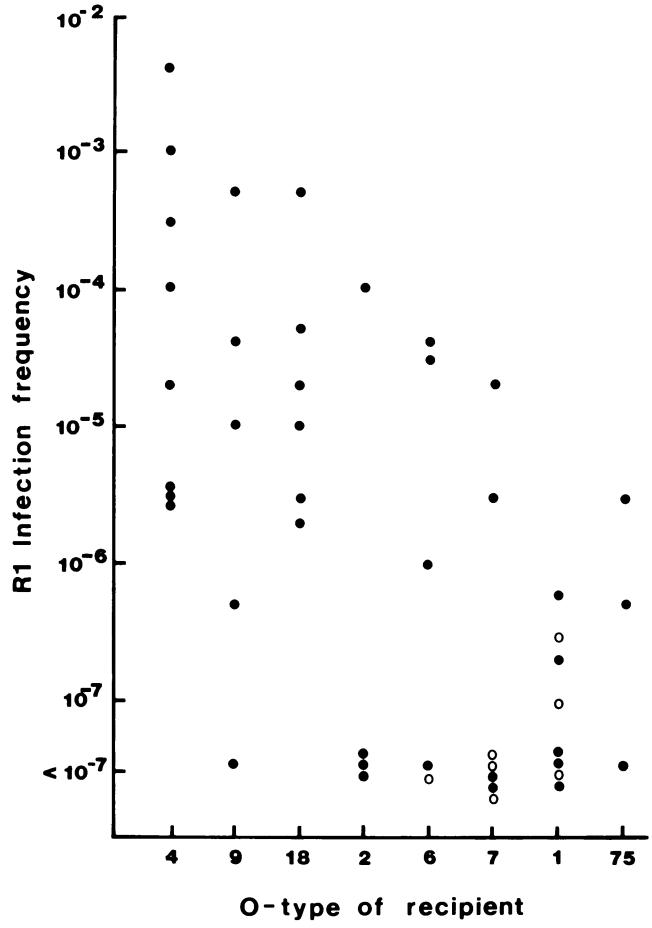

FIG. 1. *R-plasmid spread among O-typed urinary tract E. coli indicated by R1 infection frequency (final viable transconjugants/final viable count recipients) after overnight broth mating with E. coli K-12 (R1). Each point represents the mean of two independent mating experiments, and open circles indicate matings in which inhibition of donor growth was observed (10^3-fold reduction in donor/recipient ratio). Infection frequencies obtained for the control mating E. coli(R1) × E. coli K-12 M560 res⁻mod⁻, included in each batch of matings, ranged from 0.8 to 4 × 10^{-2}.*

frequencies were accompanied by a large decrease in the *E. coli* K-12 (R1) donor population (the ratio of donor to recipient was reduced from ca. 1:1 to $1:10^3$ to 10^6). The recipients in these matings, three from O-type O1, three from O7, and one from O6, were found to be colicinogenic, all producing colicin(s) of group A and three producing colicins of both groups A and B. To assess whether or not this could be a factor in R-plasmid spread, the distribution of colicinogeny among urinary tract isolates was examined. The results obtained (Table 3) indicate that, as with antibiotic resistance, colicin production is not randomly distributed among the O-types. Of particular interest was the finding that, apart from O75 isolates, there appears to be an inverse correlation between the incidence of group A colicinogeny and the incidence of antibiotic resistance. Group A colicins, when further identified with an enlarged range of indicators, were found to be mostly colicin E1, E2, E3, and A (4, 11). Neither these colicins nor the few examples of D, K, and X were clearly limited to particular O-types. Of the seven urinary isolates found to inhibit the R1-bearing *E. coli* K-12, six were remated with a colicin-insensitive variant of the donor. In every case, the high inhibition of the donor was eliminated (maximum change in the donor/recipient ratio of 50), but the R1 infection frequencies were very similar to those previously obtained. None of the six achieved an infection frequency greater than 5×10^{-7}, and four, as before, gave no detectable transfer (i.e., $<10^{-7}$). The differences between the infection frequencies attained by the various O-types therefore appear to reflect basic differences between the strains and are not simply due to inhibition of the donor by the recipient.

TABLE 3. *Colicinogeny among E. coli isolated from urinary tract infections*[a]

O-type	No. tested	No. (%) producing colicin of:	
		Group A	Group B
O4	14	1 (7)	2 (14)
O9	11	1 (9)	6 (55)
O18	22	2 (9)	5 (23)
O2	8	0 (<13)	1 (13)
O6	14	3 (21)	4 (29)
O7	14	11 (79)	1 (7)
O1	24	13 (54)	11 (46)
O75	24	2 (8)	0 (<4)

[a] Number and percentage of *E. coli* urinary tract isolates producing colicins of groups A and B as determined by action on standard indicators.

To ascertain whether differences in plasmid spread were host or plasmid determined, randomly selected non-colicinogenic recipients from the O-types O4, O18, O1, and O75 were mated with *E. coli* K-12 bearing one of the five R-plasmids of incompatibility (*inc*) groups other than FII (Table 1). The data obtained (Fig. 2A)

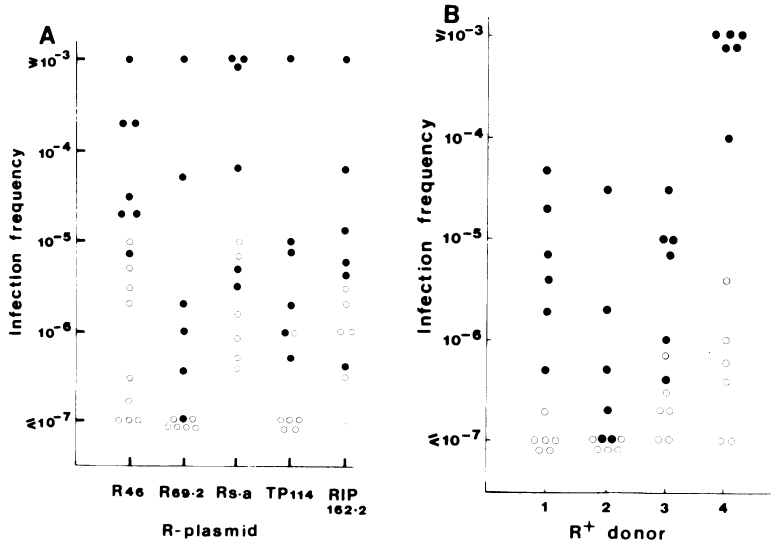

FIG. 2. *Spread of R-plasmids among O-typed urinary tract E. coli indicated by infection frequency after overnight mating in broth (R46) or on solid medium (all others). Recipients belong to O-types of either low (○, O1 and O75) or high (●, O4 and O18) incidence of antibiotic resistance (Table 2). Donors used were (Table 1): (A) E. coli K-12 strains bearing R-plasmids R46, R69-2, Rs-a, TP114, and R1P162-2 of inc groups N, M, W, I_2, and FI, respectively. (B) E. coli isolated from urinary tract infections and carrying transmissible antibiotic-resistance determinants. (1) SPA440, O-type O18; (2) SPA481, O4; (3) SPA5660, O6; (4) SPA1977, O9.*

show that infection frequency with these plasmids, as with R1, was clearly greater for strains belonging to the high-incidence O-types. These recipients were also mated with R^+ urinary tract isolates of O-types O4, O6, O9, and O18. Again, the R-plasmid infection frequencies obtained (Fig. 2B) concur with the initial observations made with the R-plasmid R1. The recipients of O-types O4 and O18 were superior to those of O-types O1 and O75.

To determine whether R-factor spread also varied when urinary tract isolates acted as donors, R1-carrying progeny of 14 strains of the high-incidence O-types and 12 strains of the low-incidence O-types were mated with a restrictionless *E. coli* K-12 recipient. In four of the matings, this strain was inhibited by donor colicin production, and these matings were therefore repeated with colicin-insensitive variants of the recipient. The results of all matings in which no inhibition was observed are shown in Fig. 3. Unlike when wild-type strains were employed as recipients, the spread of R1 from the urinary strains to a competent recipient was similar for all of the O-types tested. The high- and low-incidence strains appeared equally capable of transferring R1 to an extent sufficient to instigate widespread plasmid infection.

No significant loss (<10%) of plasmid R1 was observed when eight isolates from both high- and low-incidence O-types bearing the plasmid R1 were incubated overnight, i.e., under mating conditions. There was also no detectable difference in the batch culture growth curves of $R1^+$ and $R1^-$ isogenic pairs of these isolates. Plasmid instability or reduced host growth rate did not therefore influence our results.

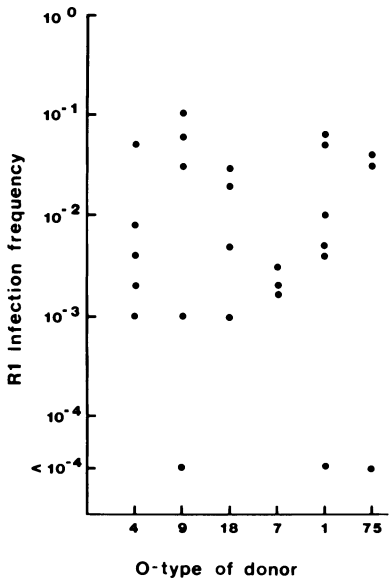

FIG. 3. *R1 spread among E. coli K-12 (res^-) during overnight mating with urinary tract E. coli carrying this plasmid. Each infection frequency is the mean of values obtained in two independent matings.*

DISCUSSION

It is apparent from our survey of *E. coli* isolates responsible for urinary tract infections at Charing Cross Hospital that the incidence of resistance to two or more antimicrobial agents has increased over the last decade and that it varies among the O-types most commonly involved in such infections. Strains of O-types O4, O7, and O18 are more frequently multiply resistant than those of other O-types, particularly O7, O1, and O75. This nonrandom distribution of antibiotic resistance among *E. coli* O-types has been reported previously for fecal strains of human and animal origin (9) and for strains isolated from various clinical sources, including urinary infections (16), but the reason for this has not been apparent. Our data now suggest two possible explanations.

The variation in the incidence of multiple-antibiotic resistance among the O-types predominant in urinary tract infections appears to reflect directly the different ability of these antigenic types to accept R-plasmids from competent hosts and establish a significant donor population capable of instigating epidemic spread. This difference is not determined by the origin of the plasmid, i.e., is not a result of superinfection immunity exerted by endogenous plasmids. Such differences in R-plasmid spread are evident not only with well-characterized plasmids of defined Inc properties but also with those currently prevalent among *E. coli* causing urinary tract infection. It seems, therefore, to be an inherent property of the host cell, possibly reflecting surface differences related directly or indirectly to O-antigen, which influences the efficiency of mating-pair or aggregate formation (1).

It also seems possible that the high incidence of colicin production among certain O-types may reduce their frequency of R-plasmid carriage by inhibiting potential donors. Our data indicate that colicins of group A are more inhibitory in broth culture than those of group B, which probably reflects differing requirements for activity of the two groups. Colicin V of group B, for example, is frequently not active on complex media such as nutrient or TSA but gives clear inhibition on media normally used for antibiotic susceptibility tests. The importance of colicinogeny in bacterial interactions is unclear (8) and, in the case of urinary tract infection, is

reduced by the high frequency of insensitivity to one or more colicins among the causative strains (10; C. Hughes, unpublished data).

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