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R-Plasmid Transfer Frequencies from Environmental Isolates of *Escherichia coli* to Laboratory and Fecal Strains

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Multiple-drug-resistant strains of *Escherichia coli* were isolated from the water at an estuarine site. They represented about 8.3% of the total *E. coli* population. Fifty-five strains, representing each of the 32 resistance patterns identified, were mated with an *E. coli* K-12 F^- strain. Matings were performed on membrane filters, and the cells were washed to remove any colicins produced by the donors. Thirty-one strains, about 5% of the mean *E. coli* density in the samples, transferred drug resistance and, hence, possessed conjugative R plasmids. Of these, 80% transferred drug resistance at a frequency of about 10^{-4} or less. Nine environmental R^+ strains were mated with three fecal recipients. The R-plasmid transfer frequencies to the fecal strains from the environmental donors correlated well with those from a derepressed K-12 R^+ laboratory donor. The $R^+ \times$ K-12 F^- *lac*$^-$ transconjugants from 16 environmental strains were "backcrossed" to a *lac*$^+$ K-12 F^- strain. All transfer frequencies were higher in the backcrosses than in the original matings from the environmental donor. Furthermore, 7 of 13 different transconjugants, which accepted plasmids at repressed frequencies of less than 10^{-3}, donated them at frequencies greater than 10^{-2}. This suggests that these were derepressed plasmids in a repressed host.

Outbreaks of food- and waterborne enteric disease caused by *Shigella* and *Salmonella* species which harbor pieces of self-replicating deoxyribonucleic acid coding for multiple drug resistance (R plasmids) have been reported in many parts of the world. That R^+ coliforms may be the source of R plasmids for these pathogens has been known since 1959, when researchers in Japan first isolated R^+ strains of *Shigella* during a dysentery outbreak and recovered *Escherichia coli* with the same resistance pattern from the same individuals. They also showed that R-plasmid exchange can occur among *E. coli*, *Shigella*, and *Salmonella* species (29).

Outbreaks of waterborne enteric disease caused by *Shigella*, *Salmonella*, or enteropathogenic *E. coli* strains which contain transferable R plasmids have rarely occurred in the United States, although the *Shigella* isolate obtained during the only known outbreak of recreational waterborne dysentery possessed multiple drug resistance (22). Nevertheless, there has been considerable concern that R plasmids in their coliform hosts are being disseminated into environmental waters via the disposal of raw or marginally disinfected sewage and sludge. The concern is that these plasmids are being transferred to enteric, bacterial pathogens in sewage, environmental waters, or individuals who harbor R^+ *E. coli* previously ingested in the course of water-associated activities, notably swimming and shellfish consumption (6, 10, 24). Because of this, some investigators have suggested the need for more stringent water quality standards limiting the densities of coliforms in environmental waters and for more advanced purification of sewage prior to its discharge into the environment (10).

The basis for concern is that multiple-drug-resistant coliforms have been isolated from sewage, its receiving waters, and sludge-dumping areas, where they comprised between 1 and 50% of the coliform population (6-9, 12, 24, 26). In addition, it has been shown that about 60% of the multiple-drug-resistant coliform isolates transfer their plasmids to laboratory strains of *E. coli*, *Salmonella*, or *Shigella* (7, 15, 26). However, in most of these studies, the actual transfer frequencies were not determined, nor were the abilities of fecal and environmental strains of *E. coli* and enteric pathogens to receive the plasmids considered. These factors are important in the assessment of risk. The present investigation was initiated to reexamine the question of waterborne dissemination of R plasmids with emphasis on these two considerations.

MATERIALS AND METHODS

Sampling and isolation procedure. Water samples were collected in January and February of 1978

from an estuarine site, Fox Point, on the Providence River in Rhode Island. The total *E. coli* concentration was determined by filtering the samples through GN-6 Metricel, 0.45-μm membrane filters (Gelman Instrument Co., Ann Arbor, Mich.) and placing the filters directly on mTEC medium (5) containing 0.25 g of indoxyl β-D-glucoside per liter. Drug-resistant *E. coli* strains were selectively isolated by incorporating chloramphenicol (Calbiochem, San Diego, Calif.), kanamycin sulfate (Calbiochem), ampicillin (Sigma Chemical Co., St. Louis, Mo.), or the combination of tetracycline hydrochloride (Sigma) and streptomycin sulfate (ICN Pharmaceuticals, Cleveland, Ohio) into the mTEC medium at concentrations of 15 μg/ml. These antibiotics were chosen because of their widespread use and importance in the treatment of gram-negative bacterial infections. Streptomycin and tetracycline were used together to increase the likelihood that resistance would be plasmid mediated rather than chromosomally mediated. The mTEC plates were incubated for 18 to 22 h at 41°C, instead of 44.5°C as described in the original procedure (5), to enhance the recovery of cells with thermosensitive plasmids (27) or thermosensitive proteins involved in determining drug resistance (13). Yellow colonies were picked and later identified as *E. coli* by the API 20 system (Analytab Products, Inc., Plainview, N.Y.).

Determination of drug resistance patterns and colicin production. The drug resistance patterns of the environmental isolates were determined from the zones of inhibition around antibiotic-impregnated disks (Difco Laboratories, Detroit, Mich.) placed on plates of Mueller-Hinton agar (GIBCO Diagnostics, Madison, Wis.) spread with the organism. The following disks were used: chloramphenicol, 10 μg; kanamycin, 10 μg, ampicillin, 10 μg; tetracylcine, 10 μg; streptomycin, 10 μg; sulfadiazine, 300 μg; and nalidixic acid, 50 μg.

Colicin production was determined by streaking the colicin-susceptible recipient strain, *E. coli* K-12 F⁻ *lac*⁻ (CSH-26), on Mueller-Hinton agar with a cotton swab and stabbing a heavy inoculum of the environmental isolate into the agar. If a zone of inhibition was seen around the stab, the strain was considered to be a colicin producer.

Estimation of R-plasmid transfer frequencies. One to three strains, representative of each resistance pattern observed among the environmental isolates, were crossed with a *lac*⁻ mutant of *E. coli* K-12 F⁻ (CSH-26), marked with resistance to nalidixic acid. The conjugation procedure was adapted from that of T. L. Corliss (M.S. thesis, University of Rhode Island, Kingston, 1979) as follows. Separate 30-ml flasks of Penassay broth (Difco) were inoculated with a few drops of overnight cultures of the donor (environmental isolate) and recipient (CSH-26) strains. When these cultures reached mid-log phase as determined by optical density, 0.1 ml of the donor culture and 0.9 ml of the recipient culture were each suspended in 10-ml volumes of Penassay broth. The donor suspension was then filtered onto a polycarbonate, 0.4-μm porosity membrane filter (Nuclepore Corp., Pleasanton, Calif.) and washed with 10 ml of liquid minimal medium E (28). Colicin-producing donor strains were washed with an additional 10 ml of medium E. The recipient suspension was then added, and the mixture was again washed with medium E. The filter was then placed on a plate of medium E for a 1-h mating period at 35°C before being transferred to the selective medium, which was incubated at 35°C for about 24 h. The selective media consisted of Mueller-Hinton agar to which were added 1.0% lactose, 0.08 g of bromothymol blue (MC&B Manufacturing Chemists, Inc., Norwood, Ohio) per liter, 50 μg of nalidixic acid (Sigma) per ml, and 10 μg of one of the following antibiotics per ml: tetracycline, chloramphenicol, kanamycin, ampicillin, or streptomycin. The pH was adjusted to 7.0–7.2 with 5 ml of 1 N NaOH per liter.

The drug resistance markers possessed by a particular donor strain were sequentially used to screen the mating mixture for R⁺ transconjugants by incorporating the particular drug in the selective medium. This was continued until transconjugants were obtained or transfer could not be detected by selecting for all the drug resistance markers possessed by the donor. The sequence in which the drugs were used was chloramphenicol, kanamycin, tetracycline, streptomycin, and ampicillin.

Low-frequency transfer was determined directly by counting the *lac*⁻ colonies on the membrane filter. For high-frequency transfers and the selection of ampicillin resistance, the membrane was removed from the medium E plate and immersed in 10 ml of buffer (30), which was then agitated with a Vortex mixer for at least 30 s. Appropriate dilutions of the suspension were spread on the selective medium. The donor concentration was determined from dilutions of the mid-log-phase culture spread on plates of the same selective medium used to select the transconjugants; however, the nalidixic acid was omitted.

Ten or more transconjugants from each mating were picked and tested for drug resistance and colicin production as described above. Very low-frequency transfers were performed in duplicate or triplicate to obtain at least 10 transconjugants and provide a better estimate of the transfer frequency.

Backcrosses and plasmid transfer to fecal strains. The *lac*⁻ transconjugants from some of the environmental strains were "backcrossed" with a *lac*⁺ *E. coli* K-12 F⁻ recipient (J5) marked with nitrofurantoin resistance. The *lac*⁺ transconjugants from these matings were selected on media which contained 80 μg of nitrofurantoin (Sigma) per ml and 10 μg of kanamycin, tetracycline, or ampicillin per ml.

Nine environmental strains which transferred drug resistance at frequencies of 10^{-4} or greater were crossed with nalidixic acid-resistant mutants of two *E. coli* strains (F-18 and F-309) and one strain of *Citrobacter freundii* (F-76) isolated from human feces by a dilution procedure. These strains accept the R1 *drd* plasmid (17, 18) from *E. coli* RS-2, at frequencies of about 10^{-7}, 10^{-1}, and 10^{-3}, respectively (T. L. Corliss, M.S. thesis, University of Rhode Island, Kingston, 1979). The selective media were the same as those used in the initial matings. Since both the donors and recipients were *lac*⁺, only the single drug marker could be used to differentiate the transconjugants from the donors. To account for mutations to nalidixic acid resistance in the donors or resistance to the plasmid-coded antibiotics in the recipients, we determined these mutation frequencies by filtering each donor and recipient alone and placing the filters on the appro-

priate selective media. The frequencies were always less than 10^{-7}, the sensitivity of the assay.

RESULTS

Occurrence of drug resistance. The total *E. coli* concentration at the sampling station was always greater than 10^4 cells per ml. The percentages of *E. coli* resistant to the various antibiotics were as follows: streptomycin, 6.7%; ampicillin, 6.2%; tetracycline, 5.0%; sulfadiazine, 5.8%; kanamycin, 3.1%; and chloramphenicol, 0.39%. The values given for tetracycline, streptomycin, and sulfadiazine are probably underestimates since the first two drugs were used together and the third was not used at all in the primary isolation media. Almost all the drug-resistant *E. coli* isolates were resistant to several antibiotics, although resistance to ampicillin appeared alone in some of them. Chloramphenicol and kanamycin resistance invariably occurred along with resistance to one or more of the other drugs (Table 1). Only one of the chloramphenicol-resistant strains was not also resistant to sulfadiazine.

Table 2 shows the percentage of environmental *E. coli* strains which possessed each of the observed resistance patterns. Although multiple resistance was not uncommon, only 32 of a possible 58 resistance patterns were represented in the 216 isolates tested. The results from other samples (data not shown) also showed that chloramphenicol-resistant strains tend to be rather infrequent and that resistance to tetracycline, streptomycin, and sulfadiazine is one of the most common patterns. About 9.2% of the total environmental *E. coli* isolates were found to be resistant to one or more of the drugs used in the study. This percentage would have been somewhat higher if tetracycline and streptomycin had been incorporated separately in the mTEC media. The percentage of multiresistant strains was 8.3% (Table 2).

Eighteen of 55 isolates (33%) produced factors (probably colicins) which killed or inhibited the growth of *E. coli* CSH-26.

Ability of environmental strains to transfer drug resistance. Table 2 also shows which resistance patterns were transferred. Of 55 strains (representing the 32 resistance patterns) examined, 31 (56%) transferred one or more of their resistance markers to *E. coli* K-12 F^- (CSH-26). These environmental R^+ strains represent about 5% of the mean *E. coli* density in

TABLE 1. *Association of drug resistance determinants with the antibiotics used for isolation*

Antibiotic(s)[a] in isolation medium	No. of strains tested	% of strains resistant to:					
		Cm	Km	Ap	Tc	Sm	Su
Cm	56	100	51.8	76.8	78.6	73.2	98.2
Km	48	0	100	52.1	81.3	85.4	62.5
Ap	57	5.3	24.6	100	52.6	63.2	57.9
Tc and Sm	55	5.5	27.3	36.4	100	100	76.4

[a] Cm, chloramphenicol; Km, kanamycin; Ap, ampicillin; Tc, tetracycline; Sm, streptomycin; Su, sulfadiazine.

TABLE 2. *Resistance patterns isolated and percentage of environmental E. coli strains with conjugative R plasmids*

Resistance pattern							% of total strains	Resistance patterns transferred[a]	
								No. positive/ no. tested	% of total strains
1.	Tc	Sm	Km	Cm	Su	Ap	0.09[b]	2/2	0.09
2.	Tc	Sm		Cm	Su	Ap	0.07[b]	0/2	0
3.	Tc			Cm	Su	Ap	0.13[b]	1/2	0.06
4.		Sm		Cm	Su		0.02	2/2	0.02
5.	Tc			Cm	Su		0.01	0/1	0
6.	Tc	Sm	Km	Cm	Su		0.03[b]	2/2	0.03
7.				Cm	Su	Ap	0.01	0/2	0
8.		Sm		Cm	Su	Ap	0.007	2/2	0.007
9.	Tc	Sm		Cm	Su		0.004	0/1	0
10.	Tc		Km	Cm	Su	Ap	0.004	1/1	0.004
11.			Km	Cm		Ap	0.004	1/1	0.004
12.			Km	Cm	Su	Ap	0.004	1/1	0.004
13.	Tc	Sm			Su		1.30	2/2	1.30
14.	Tc	Sm	Km		Su	Ap	0.48[b]	2/2	0.48
15.	Tc	Sm					0.36	1/2	0.18
16.	Tc	Sm			Su	Ap	0.69[b]	1/1	0.69
17.	Tc	Sm				Ap	0.21[b]	1/2	0.11
18.	Tc	Sm	Km				0.14[b]	1/2	0.07
19.						Ap	0.94	0/2	0
20.		Sm			Su	Ap	0.94	1/2	0.47
21.		Sm				Ap	0.59	2/2	0.59
22.		Sm	Km			Ap	0.47[b]	0/2	0
23.	Tc		Km		Su	Ap	0.28	2/2	0.28
24.	Tc					Ap	0.36	1/2	0.18
25.	Tc				Su	Ap	0.36	0/1	0
26.	Tc	Sm	Km			Ap	0.26[b]	2/2	0.26
27.					Su	Ap	0.12	0/1	0
28.	Tc	Sm	Km		Su		0.80	1/3	0.27
29.	Tc		Km		Su		0.29	0/2	0
30.		Sm	Km		Su	Ap	0.12	1/2	0.06
31.		Sm	Km				0.06	0/1	0
32.	Tc		Km			Ap	0.06	1/1	0.06
Total							9.2		5.2
Total multiresistant							8.3		

[a] Number which transferred at least two resistance markers or a single marker at a frequency appreciably higher than the mutation rate of the recipient, K-12 F^- (CSH-26), to its resistance. Drugs are abbreviated as in Table 1.

[b] The same pattern was obtained with isolates from more than one of the primary, antibiotic-containing media, and the percentage shown is an average.

the samples. The mutation frequencies of CSH-26 to resistance to the various plasmid-coded antibiotics were less than the lowest transfer frequency that was obtained (4.8×10^{-7}).

One-half of the conjugative strains tested transferred all of their resistance determinants to the F^- strain. Twenty-five (80%) of the conjugative strains consistently transferred the same markers to the K-12 F^- recipient. The number of resistance determinants transferred by the remaining 20% varied from transconjugant to transconjugant. For instance, 4 of the 10 transconjugants from the K-23 × K-12 F^- cross received the full complement of drug resistance (kanamycin, streptomycin, ampicillin, and sulfadiazine), whereas six transconjugants were resistant only to kanamycin, streptomycin, and ampicillin. The common resistance determinants, those to tetracycline and streptomycin, were also commonly transferred.

Since nine of the 18 "colicin-producing" strains carried conjugative R plasmids and one of these strains transferred resistance at the "derepressed" rate of 10^{-2} per donor cell, it is unlikely that colicin production affected a strain's ability to transfer R plasmids under the conditions used in this study. None of the strains transferred the ability to produce colicins to the F^- strain.

Although 31 strains had conjugative R plasmids in that they transferred more than one drug marker or transferred the marker at a frequency greater than the mutation frequency of the recipient, only six strains transferred drug resistance at a frequency of 10^{-3} or greater. The distribution of the environmental strains according to their conjugation frequencies is shown in Fig. 1. The frequencies used in the calculations are averages of two or more matings. Only four of the environmental R^+ strains appeared to be derepressed in that the transfer frequencies approached that of the derepressed control donor. Most of the conjugative strains were repressed and could only transfer resistance at frequencies of about 10^{-4} or less. Figure 2 is the cumulative probability plot of the R-plasmid transfer frequencies from the environmental strains to CSH-26. This graph indicates there are at least two distinct populations of R^+ *E. coli*: the "derepressed" population with transfer frequencies of 10^{-2} or greater and the repressed population with transfer frequencies of less than 10^{-3}.

Plasmid transfer to fecal strains. Nine environmental R^+ *E. coli* strains which transferred their plasmids at moderate to high frequencies (10^{-4} to 10^{-2}) were mated with three fecal strains whose abilities to accept plasmids were known. Table 3 gives the transfer frequencies and the resistance determinants transferred

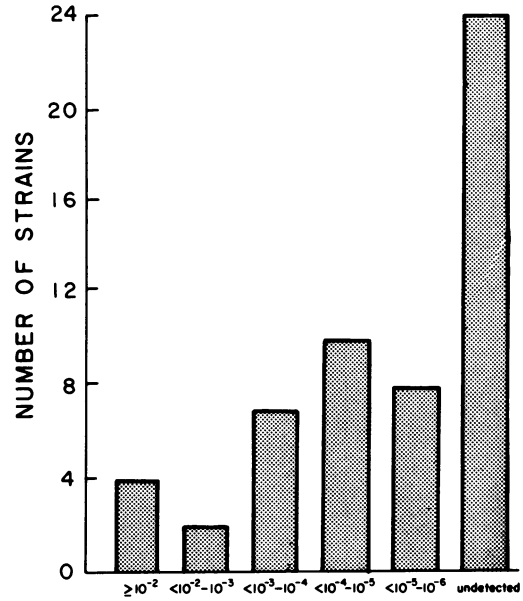

FIG. 1. *R-plasmid transfer frequencies from environmental donors to E. coli K-12 F⁻.*

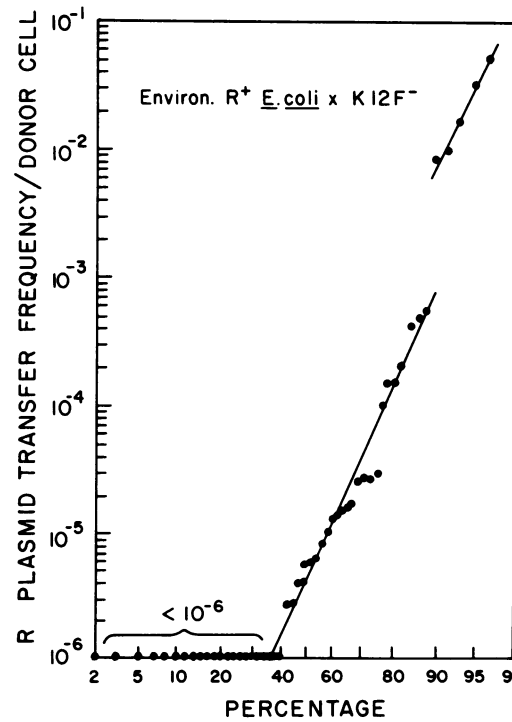

FIG. 2. *Cumulative probability plot of R-plasmid transfer frequencies from environmental donors to E. coli K-12 F⁻.*

TABLE 3. *R-plasmid transfer to fecal strains*

Donor strain's resistance	Recipient strains							
	CHS-26		F-18		F-76		F-309	
	Transfer frequency[a]	Resistance transferred[b]	Transfer frequency	Resistance transferred	Transfer frequency	Resistance transferred	Transfer frequency	Resistance transferred
C18 Cm Km Tc Sm Ap Su	1.7×10^{-4}	*Cm* Tc[c]	NTR[d]		6.8×10^{-5}	5/10 *Tc* 3/10 Cm *Tc* 1/10 *Tc* Ap 1/10 Cm *Tc* Ap	7.5×10^{-3}	2/10 Cm *Tc* 8/10 *Tc*
K-2 Km Tc Sm Ap Su	5.9×10^{-3}	*Km* Tc Sm Ap	NTR		1.3×10^{-5}	*Km* Tc Sm Ap	1.0×10^{-3}	*Km* Tc Sm Ap
K-14 Km Tc Sm Ap	4.4×10^{-4}	*Km*	NTR		1.5×10^{-4}	*Km*	2.7×10^{-4}	9/10 *Km* 1/10 *Km* Tc Sm
K-23 Km Sm Ap Su	5.1×10^{-2}	6/10 *Km* Sm Ap 4/10 *Km* Sm Ap Su	8.5×10^{-6}	*Km* Sm Ap (Su)[e]	8.1×10^{-3}	8/10 *Km* Sm Ap 1/10 *Km* Ap 1/10 *Km* Sm Sp Su	5.5×10^{-2}	*Km* Sm Ap
K-31 Km Tc Sm Ap	4.9×10^{-4}	*Km* Tc Sm Ap	NTR		6.9×10^{-7}	*Km* Tc Sm Ap	2.4×10^{-4}	*Km* Tc Sm Ap
K-36 Km Tc Ap	2.0×10^{-4}	7/10 *Tc* 3/10 *Tc* Ap	NTR		4.3×10^{-5}	6/10 *Tc* 4/10 *Tc* Ap	5.0×10^{-3}	9/10 *Tc* 1/10 *Tc* Ap
A1 Tc Sm Ap	1.7×10^{-2}	*Tc* Sm Ap	3.7×10^{-6}	*Tc* Sm Ap	3.5×10^{-2}	*Tc* Sm Ap	3.9×10^{-2}	*Tc* Sm Ap
A8 Sm Ap	1.7×10^{-4}	Sm *Ap*	NTR		1.4×10^{-3}	Ap	3.7×10^{-4}	Sm *Ap*
A47 Tc Sm Ap Su	5.3×10^{-4}	9/10 *Tc* 1/10 *Tc* Ap Su	NTR		4.4×10^{-5}	8/10 *Tc* 2/10 *Tc* Ap	9.4×10^{-3}	7/10 *Tc* 2/10 *Tc* Ap 1/10 *Tc Sm* Su
RS-2 Cm Km Sm Ap Su	2.7×10^{-2}	Cm Km Sm Ap Su	10^{-7}[f]	Cm Km Sm Ap Su	2.0×10^{-3}[f]	Cm Km Sm Ap Su	9.2×10^{-2}[f]	Cm Km Sm Ap Su

[a] Transfer frequencies per donor cell as obtained from the matings.

[b] At least 10 transconjugants were picked, and their resistance patterns were determined as described in the text. The fraction of transconjugants with each pattern is given. If no fraction is shown, then all transconjugants picked had that resistance pattern.

[c] The italicized drug was the selected plasmid marker. The abbreviations are explained in Table 1.

[d] No transconjugants recovered.

[e] F-18 is naturally resistant to sulfadiazine.

[f] T. L. Corliss, M.S. thesis, University of Rhode Island, Kingston, 1979.

from each environmental donor to K-12 F^- (CSH-26) and the three fecal strains. T. L. Corliss (M.S. thesis, University of Rhode Island, Kingston, 1979) has shown that strain F-309 accepts the R1 *drd* plasmid from a derepressed donor (RS-2) at a frequency comparable to that of K-12 F^- and that strains F-76 (*C. freundii*) and F-18 accept the R1 *drd* plasmid at frequencies two and five logs less than F-309, respectively. In general, these relationships were also observed when these strains were the recipients of naturally occurring plasmids (Table 3). The exceptions were that F-76 accepted plasmids from strains K14, A1, and A8 at frequencies comparable to or better than F-309. Beyond this, there were no interactions which altered the RS-2-fecal recipient relationships observed by Corliss, and the two donors with the highest frequencies of transfer to K-12 F^-, K23 and A1, also had the highest frequencies of transfer to the fecal strains. The proportion of resistance determinants transferred was not related to the transfer frequency.

The recipient appeared to influence the resistance determinants transferred. For example, A8 always transferred streptomycin and ampicillin resistance to CSH-26 and F-309 at a frequency of about 10^{-4}; however, it transferred ampicillin resistance alone to F-76 but at a higher frequency of 10^{-3}. Occasionally, a fecal strain acquired drug resistance from the donor that was not detected in the control recipient. Streptomycin resistance in F-309 from A47 is one example.

Transfer of naturally occurring plasmids from transconjugants. Repression of pilus formation in environmental strains could be mediated by the R plasmid, the host's chromosome, or another plasmid. To obtain an insight into which mechanisms were operative, we crossed transconjugants obtained from mating 16 R^+ environmental isolates with the *E. coli* K-12 F^- *lac*$^-$ strain (En R^+ × K-12 F^-) with the *lac*$^+$ variant of the same strain, J5. The R-plasmid transfer frequencies from the initital and subsequent matings are shown in Fig. 3. Each point on the graph was obtained from a separate mating. Each EN R^+ × K-12 F^- transconjugant transferred the plasmid from its corresponding environmental donor better than the environmental donor itself. Transconjugants from 7 of 13 strains which initially donated their plasmids at frequencies less than 10^0 "backcrossed" their plasmids at frequencies greater than 10^{-2} (see C47, K17, C56, A47, K14, K31, and K36). J5 seems to be a better recipient than CSH-26, and this may account for an increase in transfer frequency of up to a factor of five. The transconjugants from two strains, C47 and K36, were even better donors than the control strain (RS-2), transferring the R1 *drd* plasmid (17, 18) at frequencies of 1.6×10^0 to 8.0×10^{-1}. Figure 4 is the cumulative probability plot of the plasmid transfer frequencies from K-12 F^- R^+ to K-12 F^-. This plot also shows two distinct populations: one "derepressed" and the other repressed.

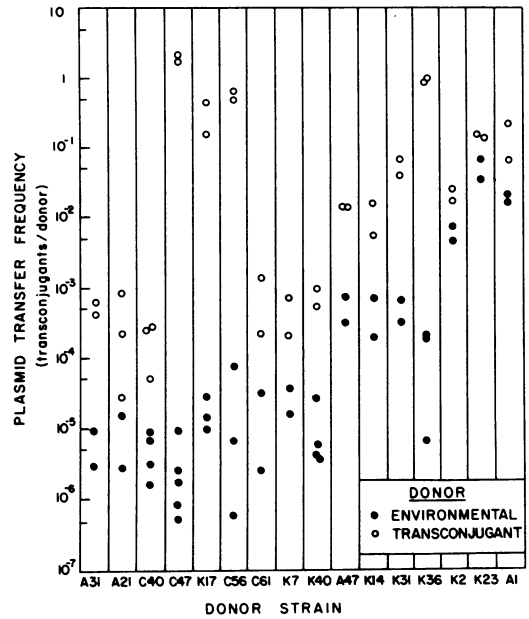

FIG. 3. *R-plasmid transfer frequencies from environmental strains and their transconjugants to K-12 F^-. The transconjugant donors were the K-12 F^- recipients of the environmental donor's R plasmid.*

DISCUSSION

The percentage of the environmental *E. coli* strains which were resistant to two or more drugs (8.3%) was in the range reported by other workers (6, 7, 9, 12, 24). Furthermore, our estimate that 60% of the multiresistant *E. coli* strains were capable of transferring all or part of their drug resistance agrees with the results for coliforms obtained by Goyal et al. (7), who reported 53%, Sturtevant and Feary (26), who reported 50%, Linton et al. (15), who reported 61%, and Grabow and Middendorff (8), who reported 34 to 41%. These percentages notwithstanding, the majority of the strains tested in this study donated their plasmids at frequencies of less than 10^{-4} per donor cell, and it is the number of donor, rather than recipient, cells which will be limiting under natural conditions in the gastrointestinal tract.

There appear to be a number of factors which

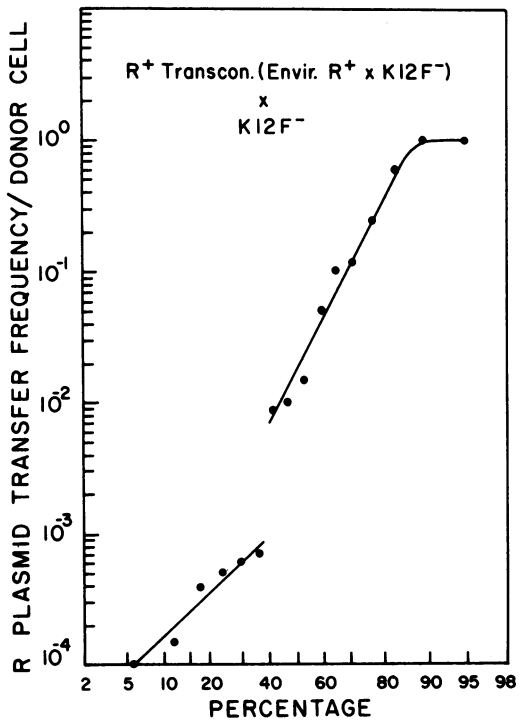

FIG. 4. *Cumulative probability plot of R-plasmid transfer frequencies from K-12 F⁻ R⁺ to K-12 F⁻.*

contribute to the low transfer frequencies obtained with most of the environmental strains. The consistently higher transfer frequencies obtained when the En R^+ × K-12 F^- transconjugants were "backcrossed" with the K-12 F^- recipient can be explained in at least two ways. The *lac*$^+$ K-12 F^- recipient (J5) used in the "backcrosses" may be a better recipient than the *lac*$^-$ K-12 F^- strain (CSH-26) used in the forward crosses. A more intriguing explanation is that contact and adherence between the mating pairs was better with the variants of the same strain in the "backcross" experiments (K-12 F^- R^+ × K-12 F^-) than between the environmental donors and laboratory K-12 F^- recipient in the forward crosses. Coupled with this explanation is the possibility that contact between the recipient and pili produced by environmental strains is prevented by factors beyond recognized repression, possibly as a result of hindrance by extracellular polymers (4).

The marked improvement in transfer frequency shown by some strains requires yet another explanation. One possibility is that the specific donor cells in the strains involved (C47, C56, K14, K17, K31, K36, A47) were mutants with host-modified deoxyribonucleic acid against the restriction endonucleases in K-12 F^-. However, this does not explain why similar mutants were not obtained with other strains. A second, more likely, explanation is that the specific plasmids which were donated at high frequencies in the "backcross" experiments were derepressed for pilus formation. The repression of pilus formation exhibited by the environmental strain could be due to a repressor of pilus formation which is coded for by chromosomal genes or by genes on a nontransferable plasmid. These plasmids could also be derepressed mutants of the normally repressed environmental plasmid.

Some of the strains which transferred only part of their resistance determinants may possess nontransferable plasmids which code for the nontransferred resistance markers. An indication of the existence of two compatible R plasmids within the same environmental strain was obtained from the results of the A8 crosses with the fecal and laboratory recipients. Ampicillin resistance alone was transferred to F-76, but it was transferred at a higher frequency than was streptomycin and ampicillin resistance to CSH-26 and F-309. The most likely explanation is that A8 has a plasmid coding for ampicillin resistance and one coding for streptomycin resistance. The latter was not detected in F-76 because it was transferred at a frequency of 10^{-4}. It was detected in CSH-26 and F-309 because they restricted the plasmid coding for ampicillin resistance. There are a number of possible explanations for the transfer patterns observed with C18. One is that chloramphenicol and tetracycline resistance are on the same plasmid but that the chloramphenicol resistance gene is not passed in most instances. An alternative explanation is that chloramphenicol resistance is on a nonconjugative plasmid which is mobilized by the one containing the tetracycline resistance marker. It is also possible that the nontransferred markers were lost because of host restriction. All of these possibilities could be used to explain the infrequent transfer of ampicillin resistance.

The relative abilities of the three fecal isolates and the K-12 F^- laboratory strain to receive R plasmids from environmental donors were generally consistent with that observed with the derepressed K-12 donor (RS-2). Resistance of the plasmid deoxyribonucleic acid to the restriction endonucleases of the *C. freundii* strain, F-76, could explain the relatively high frequencies observed with the three exceptions (K14, A1, and A8 when mated with F-76).

R-plasmid transfer frequencies obtained in this study do not appear to support the proposal

for more stringent water quality criteria for colforms as suggested by Grabow et al. (10). It has been estimated that an individual swimming in barely acceptable waters may accidentally ingest between one and five R^+ coliforms on the basis of our data and those of Grabow et al. (10). About 60% of R^+ strains did possess conjugative R plasmids; however, the majority of these strains transferred their plasmids at frequencies of 10^{-4} or less per donor cell. Therefore, the likelihood that one of one to five ingested *E. coli* will donate its resistance to the fecal flora becomes minimal, especially since fecal strains are generally poor recipients. The probability that an R plasmid from the ingested R^+ *E. coli* will be transferred to a resident *E. coli* or a subsequently ingested enteric pathogen could be increased if the R^+ *E. coli* colonized the bowel of the individual and thereby increased its numbers. Plasmid transfer between *E. coli* strains can occur in the gut (23), even in the absence of antibiotics (19, 31), although this is a rare phenomenon (21). Usually, though, more than 10^6 bacteria must be ingested in order for colonization to occur (25), and even when 10^{10} viable organisms are ingested, colonization by R-plasmid-bearing strains is poor (1). Furthermore, if R-plasmid transfer between an ingested R^+ coliform and a resident *E. coli* strain does occur, there is a question as to how long the R^+ transconjugant will remain in the bowel (1, 14, 19, 25). This period of time will influence the probability that the R^+ bacteria will transfer resistance to a subsequently ingested enteric pathogen.

Anderson has shown that *Bacteroides fragilis* inhibits plasmid transfer among *E. coli* strains (2). It is now known that *Bacteroides* spp. harbor transmissible R plasmids (11, 16, 20) and can, under certain conditions, receive plasmids from *E. coli* (3). Since *Bacteroides* spp. are at least a thousand times more numerous in the fecal flora than *E. coli*, it seems that they would be the most likely recipients of and subsequent reservoir for an R plasmid donated by *E. coli*. If this is true, then plasmid transfer may be more common in vivo than has been reported in the past.

Because of the current standards for permissible coliform levels at bathing beaches, the low frequency of piliated cells, the concentrations required for colonization, and the rarity of plasmid transfer in the human alimentary tract, the "health risk" to swimmers posed by R-plasmid-bearing coliforms does not seem to be great. However, this does not mean that we should not be concerned about the increasing number of R-plasmid-carrying strains.

LITERATURE CITED

1. **Anderson, J. D.** 1974. The effect of R factor carriage on the survival of *Escherichia coli* in the human intestine. J. Med. Microbiol. **7**:85–90.
2. **Anderson, J. D.** 1975. Factors that may prevent transfer of antibiotic resistance between gram-negative bacteria in the gut. J. Med. Microbiol. **8**:83–88.
3. **Burt, S. J., and D. R. Woods.** 1976. R factor transfer to obligate anaerobes from *Escherichia coli*. J. Gen. Microbiol. **93**:405–409.
4. **Cagle, G. D.** 1975. Fine structure and distribution of extracellular polymer surrounding selected aerobic bacteria. Can. J. Microbiol. **21**:395–408.
5. **Dufour, A. P., E. R. Strickland, and V. J. Cabelli.** 1975. Enumeration method for thermotolerant *E. coli*, p. 79–81. *In* D. S. Wilt (ed.), Proceedings of the National Shellfish Sanitation Workshop, 25 June 1975; Charlestown, S.C. U.S. Food and Drug Administration, Washington, D.C.
6. **Feary, T. W., A. B. Sturtevant, and J. Lankford.** 1972. Antibiotic resistant coliforms in fresh and salt water. Arch. Environ. Health **25**:215–220.
7. **Goyal, S. M., C. P. Gerba, and J. L. Melnick.** 1979. Bacteria in estuarine sediments. Mar. Pollut. Bull. **10**: 25–27.
8. **Grabow, W. O. K., and I. G. Middendorff.** 1973. Survival in maturation ponds of coliform bacteria with transferable drug resistance. Water Res. **7**:1589–1597.
9. **Grabow, W. O. K., O. W. Prozesky, and J. S. Burger.** 1975. Behavior in a river and dam of coliform bacteria with transferable or non-transferable drug resistance. Water Res. **9**:777–782.
10. **Grabow, W. O. K., O. W. Prozesky, and L. S. Smith.** 1974. Drug resistant coliforms call for a review of water quality standards. Water Res. **8**:1–9.
11. **Guiney, D. G., and C. E. Davis.** 1978. Identification of a conjugative R plasmid in *Bacteroides ochraceus* capable of transfer to *Escherichia coli*. Nature (London) **274**:181–182.
12. **Kelch, W. J., and J. S. Lee.** 1978. Antibiotic resistance patterns of gram-negative bacteria isolated from environmental sources. Appl. Environ. Microbiol. **36**:450–456.
13. **Levy, S. B., and L. McMurry.** 1974. Detection of an inducible membrane protein associated with R-factor-mediated tetracycline resistance. Biochem. Biophys. Res. Commun. **56**:1060–1068.
14. **Linton, A. H., K. Howe, P. M. Bennett, and M. H. Richmond.** 1977. The colonization of the human gut by antibiotic resistant *Escherichia coli* from chickens. J. Appl. Bacteriol. **43**:465–469.
15. **Linton, K. B., P. A. Lee, M. H. Richmond, W. A. Gillespie, A. J. Rowland, and V. N. Baker.** 1972. Antibiotic resistance and transmissible R-factors in the intestinal flora of healthy adults and children in an urban and rural community. J. Hyg. **70**:99–104.
16. **Mancini, C., and R. J. Behme.** 1977. Transfer of multiple antibiotic resistance from *Bacteroides fragilis* to *Escherichia coli*. J. Infect. Dis. **36**:597–600.
17. **Meynell, E., and N. Datta.** 1967. Mutant drug resistant factors of high transmissibility. Nature (London) **214**: 885–887.
18. **Meynell, E., G. G. Meynell, and N. Datta.** 1968. Phylogenic relationships of drug resistance factors and other transmissible bacterial plasmids. Bacteriol. Rev. **32**:55–83.
19. **Petrocheilou, V., J. Grinsted, and M. H. Richmond.** 1976. R-plasmid transfer in vivo in the absence of antibiotic selection pressure. Antimicrob. Agents Chemother. **10**:753–761.
20. **Privitera, G., M. Sebald, and F. Fayolle.** 1979. Com-

mon regulatory mechanisms of expression and conjugative ability of a tetracycline resistance plasmid in *Bacteroides fragilis*. Nature (London) **278**:657-658.

21. **Richmond, M. H., and V. Petrocheilou.** 1978. R-factor transfer in vivo in humans, p. 273-276. *In* D. Schlessinger (ed.), Microbiology—1978. American Society for Microbiology, Washington, D.C.
22. **Rosenberg, M. L., K. K. Hazlet, J, Schaefer, J. G. Wells, and R. C. Pruenda.** 1976. Shigellosis from swimming. J. Am. Med. Assoc. **236**:1849-1852.
23. **Smith, H. W.** 1969. Transfer of antibiotic resistance from animal and human strains of *Escherichia coli* to resident *E. coli* in the alimentary tract of man. Lancet **1**: 1174-1176.
24. **Smith, H. W.** 1971. Incidence of R^+ *Escherichia coli* in coastal bathing waters of Britain. Nature (London) **234**: 155-156.
25. **Smith, H. W.** 1975. Survival of orally administered *E. coli* K12 in the alimentary tract of man. Nature (London) **255**:500-502.
26. **Sturtevant, A. B., and T. W. Feary.** 1969. Incidence of infectious drug resistance among lactose fermenting bacteria isolated from raw and treated sewage. Appl. Microbiol. **18**:918-924.
27. **Terawaki, Y., H. Takayasu, and T. Akiba.** 1967. Thermosensitive replication of a kanamycin resistance factor. J. Bacteriol. **94**:687-690.
28. **Vogel, H. J., and D. M. Bonner.** 1956. Acetylornithinase of *Escherichia coli*: partial purification and some properties. J. Biol. Chem. **218**:97-106.
29. **Watanabe, T.** 1963. Infective heredity of multiple drug resistance in bacteria. Bacteriol. Rev. **27**:87-115.
30. **Watkins, W. D., C. D. Thomas, and V. J. Cabelli.** 1976. Membrane filter procedure for enumeration of *Vibrio parahaemolyticus*. Appl. Environ. Microbiol. **32**:679-684.
31. **Williams, P. H.** 1977. Plasmid transfer in the human alimentary tract. FEMS Microbiol. Lett. **2**:91-95.