

Incidence of Infectious Drug Resistance Among Lactose-Fermenting Bacteria Isolated from Raw and Treated Sewage

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Received for publication 25 August 1969

Raw and treated sewage samples were examined for antibiotic-resistant, lactose-fermenting bacteria. Approximately 1% of the total lactose-fermenting bacteria were multiply resistant. Of these organisms, 50% were capable of transferring all or part of their resistance to a drug-sensitive recipient. Only 43% of those isolated on media containing a single antibiotic were capable of resistance transfer, whereas 57% of those recovered on multiple antibiotic plates transferred resistance. R factors conferring resistance to chloramphenicol, streptomycin, and tetracycline; streptomycin and tetracycline; and ampicillin, streptomycin, and tetracycline accounted for 22, 19, and 15%, respectively, of those identified. The data indicate a significant level of infectious drug resistance among the intestinal bacteria of the urban population.

Since its discovery in Japan in 1959 (10), infectious drug resistance, mediated by episomal elements known as R factors, has been shown to be an important factor in the spread of multiple antibiotic resistance among all members of the *Enterobacteriaceae* as well as to unrelated gram-negative bacteria such as *Pseudomonas aeruginosa*, *Vibrio cholerae*, and *Pasteurella pestis* (7). The major selective force favoring the emergence of drug-resistant bacteria is antibiotic usage (5). Thus, it is not surprising that the wide distribution and high incidence of R factors among gram-negative bacteria have been noted mainly among clinical isolates associated with human and animal disease (1). Datta (2) recently estimated that approximately 50% of all clinically isolated gram-negative potential pathogens are resistant to one or more antibiotics and that this resistance is largely determined by R factors. Currently, there is little information available on the incidence of resistance and of R factors among nonclinical isolates of gram-negative bacteria.

This study was undertaken to assess the incidence of antibiotic resistance and of R factors among lactose-fermenting organisms isolated from raw and treated sewage. Since R factors have been identified among the gram-negative intestinal flora of presumably healthy individuals (2, 8), their detection and characterization among bacteria found in sewage could serve as an indi-

cation of the level of infectious drug resistance existing in the general population of the community.

MATERIALS AND METHODS

Sewage samples. Duplicate grab samples of both influent and effluent sewage were obtained from five sewage treatment plants in Jefferson County, Alabama, on two different occasions. All samples were collected in sterile bottles and processed in the laboratory within 3 hr of their collection in the field.

Isolation of resistant bacteria. All sewage samples were serially diluted in TM buffer [1.21 g of tris (hydroxymethyl)aminomethane, 8.75 g of NaCl, and 2.47 g of $MgSO_4 \cdot 7H_2O$, per liter of distilled water adjusted to pH 7.1 with HCl]. Appropriate dilutions in duplicate 0.1-ml portions were plated onto plain MacConkey Agar (BBL) to obtain estimates of the total number of lactose-fermenting bacteria. Estimates of the number of antibiotic-resistant lactose fermenters in each sewage sample were obtained by plating duplicate 0.1-ml portions of suitable dilutions onto MacConkey Agar containing the following antibiotics, separately or in combination: gentamicin, 10 μ g/ml; chloramphenicol, 5 μ g/ml; dihydrostreptomycin, 10 μ g/ml; tetracycline, 5 μ g/ml; and ampicillin, 10 μ g/ml. Lactose-fermenting colonies growing on antibiotic-containing MacConkey Agar were picked to 1 ml of TM buffer and restreaked onto MacConkey Agar containing the same antibiotic as the medium from which the original isolate was made for pure colony isolation. A single, well-isolated colony was then inoculated to a Kligler Iron Agar (BBL) slant

which, after overnight incubation at 37 C, served as a stock culture. Each isolate was subsequently identified by the methods outlined by Edwards and Ewing (3).

Antibiotic sensitivity testing. Drug resistance patterns of all bacterial strains were determined by spreading 0.1 ml of a 3- to 4-hr broth culture of the organism to be tested onto drug-free Brain Heart Infusion Agar. Sensi-discs (BBL) were dispensed onto the surface of the seeded plates. After incubation at 37 C for 18 to 24 hr, plates were examined for areas of growth inhibition surrounding the discs. The following antibiotics were used to determine patterns of resistance: ampicillin, 10 μ g; chloramphenicol, 5 μ g; cephalothin, 30 μ g; colistin, 10 μ g; dihydrostreptomycin, 10 μ g; gentamicin, 10 μ g; kanamycin, 30 μ g; nalidixic acid, 5 μ g; and tetracycline, 5 μ g.

Transfer of antibiotic resistance. Lactose-positive sewage isolates found to be resistant to one or more antibiotics were used as prospective donors of resistance to a completely antibiotic-sensitive F-derivative of *Escherichia coli* K-12. This recipient strain, designated WI-A2 (*lac*⁻), is a lactose-negative mutant of strain WI-A2 which is resistant to 250 μ g sodium azide per ml (6). Mating procedures were carried out by mixing 0.1 and 0.2 ml of overnight broth cultures of the prospective donor and recipient, respectively, in 2 ml of sterile broth. The mixtures were incubated at 37 C for 18 to 24 hr. After mixed growth, a heavy loopful of each mixture was smeared onto MacConkey Agar plates containing 250 μ g of sodium azide per ml and a single appropriate antibiotic. In this manner, 12 mating mixtures could be spotted on each plate. The media used were selective for antibiotic-resistant recombinants of WI-A2 (*lac*⁻), since growth of the prospective donor was prevented by sodium azide and growth of the recipient was prevented by an antibiotic. After incubation at 37 C for 48 hr, lactose-negative recombinants were restreaked to the same selective medium to ensure pure colony isolation. The antibiotic resistance pattern of at least one recombinant colony from each mixture was determined as described above to ascertain whether all or only a portion of the drug resistance pattern of the donor had been transferred to the recipient.

Antibiotics. Chloramphenicol was provided by Parke, Davis & Co., ampicillin (Penbritin) was supplied by Ayerst Laboratories, and gentamicin (Garamycin) was provided by the Schering Corp. Appropriate concentrations of each antibiotic used in selective media were prepared in sterile distilled water, and stock solutions were maintained by storage at -10 C.

RESULTS

Selective media containing a single antibiotic.

The numbers of lactose-fermenting bacteria from raw and treated sewage obtained from five sewage treatment plants and the incidence of bacteria resistant to either streptomycin, tetracycline, or chloramphenicol are presented in Table 1. The total number of lactose-fermenting bacteria was relatively consistent from one plant to another and there was no significant difference between

TABLE 1. Selection of antibiotic-resistant, lactose-fermenting bacteria from raw and treated sewage on MacConkey Agar containing single antibiotics

Sample	No. of lactose-positive colonies/ml			
	No anti-biotic	Strepto-mycin	Tetra-cycline	Chloram-phenicol
BI-1 ^a	10 ⁶	5 × 10 ³	10 ³	<10
BI-2	10 ⁶	5 × 10 ³	5 × 10 ³	2 × 10 ¹
BE-1	5 × 10 ⁵	3 × 10 ⁴	3 × 10 ⁴	8 × 10 ²
BE-2	10 ⁶	>10 ⁶	3 × 10 ⁴	10 ³
CI-1	5 × 10 ⁶	2 × 10 ³	3 × 10 ⁴	3 × 10 ³
CI-2	10 ⁶	2 × 10 ⁴	10 ⁴	5 × 10 ²
CE-1	10 ⁵	4 × 10 ²	8 × 10 ⁴	<10
CE-2	10 ⁵	5 × 10 ²	2 × 10 ³	<10
DI-1	10 ⁶	3 × 10 ⁴	10 ⁴	<10
DI-2	10 ⁵	5 × 10 ⁴	10 ⁴	10 ³
DE-1	10 ⁵	5 × 10 ³	3 × 10 ²	<10
DE-2	10 ⁵	10 ³	2 × 10 ³	<10
EI-1	4 × 10 ⁵	5 × 10 ⁴	5 × 10 ⁴	10 ³
EI-2	7 × 10 ⁵	5 × 10 ⁴	5 × 10 ⁴	5 × 10 ²
EE-1	10 ⁶	5 × 10 ³	8 × 10 ⁴	8 × 10 ²
EE-2	5 × 10 ⁵	>10 ⁶	5 × 10 ³	5 × 10 ²
FI-1	5 × 10 ⁶	10 ⁵	5 × 10 ⁴	7 × 10 ¹
FI-2	10 ⁶	10 ⁵	3 × 10 ⁴	2 × 10 ³
FE-1	2 × 10 ⁶	>10 ⁶	>10 ⁶	3 × 10 ²
FE-2	5 × 10 ⁶	>10 ⁶	>10 ⁶	5 × 10 ³

^a For abbreviations: the first letter designates the particular sewage disposal plant; I designates influent and E, effluent; 1 and 2 are duplicate samples.

the numbers present in the raw influents and those present in the treated effluents from these plants. The incidence of lactose-fermenting bacteria resistant to streptomycin or tetracycline varied from 0.01 to 1% of the total, whereas the incidence of lactose fermenters resistant to chloramphenicol was found to be about 10- to 100-fold less than this. There was no significant difference in the incidence of drug-resistant bacteria in raw or treated sewage. No lactose-positive colonies were isolated on media containing 10 μ g of gentamicin per ml. On all of the antibiotic selective media, the number of lactose-negative colonies observed was 10- to 50-fold greater than the number of lactose-positive colonies. On media containing gentamicin, approximately 10² to 10³ lactose-negative colonies per ml were observed. Drug-resistant lactose-negative colonies were not studied further.

From the selective media containing either streptomycin, tetracycline, or chloramphenicol, a total of 118 lactose-positive colonies were picked and purified by restreaking to a second set of selective plates. Of these, 106 were found to be *E. coli*, whereas 12 were designated *E. intermedia*

TABLE 2. Incidence and transferability of resistance patterns among *Escherichia* species selected on MacConkey Agar containing single antibiotics

Strain	Antibiotic used for selection ^a								
	Streptomycin	No.	Transferred resistance	Tetracycline	No.	Transferred resistance	Chloramphenicol	No.	Transferred resistance
<i>E. coli</i>	Am, Ds	6	0	Ds, Te	16	8(4)	C, Ds, Te	10	10
	Ds, Te	4	2(2) ^b	Am, Ds, Te	14	6(2)	Am, C	6	0
	Am, Cf, Ds	4	0	Am, Te	6	(2)	C, Te	4	2
	Other patterns ^c	14	2(2)	Am, Cf, Ds, Te	6	0	Other patterns	4	0
				Te	4	0			
			Other patterns	8	(4)				
Total		28	4(4)		54	14(12)		24	12
<i>E. intermedia</i>	Am, Ds	2	0	Am, Cf, Te	4	0			
	Other patterns	3	(1)	Other patterns	3	1			
Total		5	(1)		7	1			

^a Abbreviations: Am, ampicillin; C, chloramphenicol; Cf, cephalothin; Ds, dihydrostreptomycin; Te, tetracycline.

^b Numbers given in parentheses indicate that only a portion of the resistance pattern was transferred.

^c In addition to multiple resistance to Am-C-Ds-Te, these patterns include resistance to cephalothin, kanamycin, or nalidixic acid.

because of their ability to utilize citrate and their failure to produce H₂S (Bergey's Manual, 7th ed.). The drug-resistance patterns of each of the 118 isolates were determined by using nine different antibiotics. The most common drug-resistance patterns observed and the number of resistant strains capable of transferring all or a part of their resistance pattern to a drug-sensitive recipient are shown in Table 2. With the exception of four strains that were found to be resistant to tetracycline alone, all of the strains examined were resistant to two or more antibiotics. The most common resistance patterns observed included various combinations of resistance to ampicillin, chloramphenicol, streptomycin, and tetracycline. In addition to multiple resistance against various combinations of these four antibiotics, 46 of the strains showed additional resistance to cephalothin, kanamycin, or nalidixic acid. None of the strains was found to be resistant to colistin or gentamicin. The strains isolated on selective media containing streptomycin exhibited the greatest diversity of resistance patterns and, at the same time, the lowest incidence of transfer; only 10 of the 33 strains tested were capable of transferring all or part of their resistance to the drug-sensitive recipient. On the other hand, 39

of the 85 strains isolated on media containing either tetracycline or chloramphenicol were capable of transferring resistance to the sensitive recipient.

Selective media containing multiple antibiotics. The results presented in Tables 1 and 2 suggested to us that the incidence of multiply resistant bacteria in sewage was such that such bacteria could easily be detected by the use of selective media containing more than one antibiotic. Consequently, raw sewage and treated sewage from four of the five sewage treatment plants were sampled a second time; appropriate dilutions were plated onto plain MacConkey Agar for an estimate of total lactose-positive bacteria and onto three selective media containing streptomycin and tetracycline and, in addition to these two antibiotics, either ampicillin or chloramphenicol.

The incidence of multiply resistant lactose-positive bacteria relative to the total number of lactose-positive bacteria in treated and raw sewage is shown in Table 3. Again, it was found that, in general, there were no significant differences either in the total numbers or numbers of multiply drug-resistant, lactose-positive bacteria from one treatment plant to another or between raw and treated sewage from these plants. The

TABLE 3. Selection of antibiotic-resistant, lactose-fermenting bacteria from raw and treated sewage on MacConkey Agar containing multiple antibiotics

Sample	No. of lactose-positive colonies/ml			
	No anti-biotic	Ds + Te ^a	Am + Ds + Te	C + Ds + Te
CI-1 ^b	2 × 10 ⁶	2 × 10 ⁴	2 × 10 ⁴	6 × 10 ¹
CI-2	4 × 10 ⁶	3 × 10 ⁴	2 × 10 ⁴	9 × 10 ¹
CE-1	10 ⁶	10 ³	10 ³	5 × 10 ¹
CE-2	3 × 10 ⁶	3 × 10 ³	2 × 10 ³	2 × 10 ¹
DI-1	3 × 10 ⁶	4 × 10 ³	4 × 10 ³	2 × 10 ³
DI-2	10 ⁶	8 × 10 ³	2 × 10 ³	7 × 10 ¹
DE-1	10 ⁶	5 × 10 ³	10 ³	10 ¹
DE-2	4 × 10 ⁶	10 ³	10 ²	10 ¹
EI-1	2 × 10 ⁶	5 × 10 ³	2 × 10 ³	3 × 10 ³
EI-2	8 × 10 ⁶	5 × 10 ³	5 × 10 ³	10 ³
EE-1	10 ⁶	2 × 10 ⁶	2 × 10 ³	2 × 10 ³
EE-2	7 × 10 ⁴	10 ²	10 ²	10 ³
FI-1	5 × 10 ⁶	8 × 10 ³	10 ⁴	3 × 10 ³
FI-2	6 × 10 ⁶	10 ⁴	5 × 10 ³	2 × 10 ³
FE-1	10 ⁶	2 × 10 ⁴	4 × 10 ³	10 ³
FE-2	2 × 10 ⁶	2 × 10 ⁴	5 × 10 ³	2 × 10 ³

^a See footnote a, Table 2.

^b See footnote a, Table 1.

numbers of lactose-positive colonies selected by streptomycin and tetracycline and by the combinations of ampicillin, streptomycin, and tetracycline were similar and were found to be approximately 0.01 to 1% of the total number of lactose-positive colonies found in the absence of antibiotics. When chloramphenicol was used in combination with streptomycin and tetracycline, a 10- to 100-fold reduction in the number of resistant colonies, similar to that seen when chloramphenicol alone was used for selection, was observed.

From the selective plates containing multiple antibiotics, a total of 144 lactose-positive colonies were picked and purified by restreaking to a second set of selective media. Subsequent characterization of each of these isolates revealed that 137 were strains of *E. coli* and that the remaining 7 isolates were identified as *Klebsiella* (2 strains), *Citrobacter* (2 strains), and *Enterobacter* (3 strains). The drug-resistance patterns of all of the 144 isolates were then determined against nine different antibiotics, and each strain was grown in mixed culture with the drug-sensitive recipient to assay for resistance transfer (Table 4). It was found that 66 of these strains were multiply

TABLE 4. Incidence and transferability of resistance patterns among isolates selected on MacConkey Agar containing multiple antibiotics

Isolate	Antibiotic combination used for selection ^a								
	Ds + Te	No.	Transferred resistance	Am + Ds + Te	No.	Transferred resistance	C + Ds + Te	No.	Transferred resistance
<i>E. coli</i>	Ds, Te ^b	19	8(2) ^c	Am, Ds, Te	32	8(4)	C, Ds, Te	15	13(1)
	Am, Ds, Te	13	3(7)	Am, Cf, Ds, Te	15	1(5)	Am, C, Ds, Te	12	4(5)
	Am, Cf, Ds, Te	6	(2)	Other patterns	2	(1)			
	Ds, K, Te	5	2(1)				C, Ds, K, Te	5	3(1)
	Am, Ds, K, Te	3	(3)				Am, C, Ds, K, Te	2	(1)
	Other patterns	2	(1)				Am, C, Cf, Ds, K, Te	2	(1)
Total		48	13(16)		49	9(10)	Other patterns	4	1
<i>Citrobacter</i>	Am, Ds, Te	1	(1)				C, Ds, NA, Te	1	0
	Ds, Te	1	1				Various patterns	4	1(1)

^a See footnote a, Table 2.

^b Abbreviations: Am, ampicillin; C, chloramphenicol; Cf, cephalothin; Ds, dihydrostreptomycin; K, kanamycin; NA, nalidixic acid; Te, tetracycline.

^c See footnote b, Table 2.

resistant only to the antibiotics included in the selective media used for isolation, and the remaining 78 strains were additionally resistant to one, two, or three antibiotics not included in the selective media. Again, none of the strains examined was found to be resistant to colistin or gentamicin. The strains isolated on media containing chloramphenicol, streptomycin, and tetracycline were found to exhibit the highest frequency of infectious drug resistance, whereas those isolated on media containing ampicillin, streptomycin, and tetracycline not only exhibited the lowest frequency of transfer but also the least variation in resistance patterns.

Resistance patterns and transferability. A summary of the incidence and transferability of resistance patterns of the isolates obtained by selection on both single and multiple antibiotic media is presented in Table 5. It is evident that the use of a single antibiotic as a selective device leads to the isolation of strains which tend to exhibit a wide variety of resistance patterns with a degree of resistance generally not as extensive as that exhibited by strains isolated on selective media containing multiple antibiotics. Only 43%

of the strains selected by a single antibiotic were capable of transferring all or part of their resistance to a sensitive recipient, whereas 57% of those selected by multiple antibiotics were capable of transfer. Regardless of whether single or multiple antibiotics were used for selection, strains exhibiting certain resistance patterns tended to transfer the entire block of resistance with high frequency. For example, 93% of the strains exhibiting the pattern chloramphenicol-dihydrostreptomycin-tetracycline transferred this pattern as a block to the sensitive recipient; 46% of those exhibiting the pattern dihydrostreptomycin-tetracycline and 29% of those exhibiting the pattern ampicillin-dihydrostreptomycin-tetracycline were capable of complete transfer. Overall, 130, or 50%, of the 262 antibiotic-resistant strains examined were found to transfer all or part of their resistance to the drug-sensitive recipient regardless of their patterns of resistance.

Of the 130 resistant strains found to be infectious resistant, 56, or 43%, were found to transfer only part of their resistance to the sensitive recipient. Donor patterns most frequently observed to be partially transferred and

TABLE 5. Summary of incidence and transferability of resistance patterns of isolates

Strain	Single antibiotics			Multiple antibiotics		
	Patterns	No.	Transferred resistance	Patterns	No.	Transferred resistance
<i>E. coli</i>	Ds, Te ^a	20	10(6) ^b	Am, Ds, Te	45	11(11)
	Am, Ds, Te	14	6(2)	Am, Cf, Ds, Te	21	1(7)
	C, Ds, Te	10	10	Ds, Te	19	8(2)
	Am, C	6	0	C, Ds, Te	15	13(1)
	Am, Ds	6	0	Am, C, Ds, Te	12	4(5)
	Am, Te	6	(2)	Ds, K, Te	5	2(1)
	Am, Cf, Ds, Te	6	0	C, Ds, K, Te	5	3(1)
	Te	4	0	Am, Ds, K, Te	3	(3)
	C, Te	4	2	Am, C, Ds, K, Te	2	(1)
	Am, Cf, Ds	4	0	Am, C, Cf, Ds, K, Te	2	(1)
	Other patterns	26	2(6)	Other patterns	8	1(2)
Total		106	30(16) ^c		137	43(35) ^c
<i>E. intermedia</i>	Am, Cf, Te	4	0	Various patterns ^d	7	2(2)
	Am, Ds	2	0			
	Other patterns	6	1(1)			
Total		12	1(1) ^c		7	2(2) ^c

^a See footnote b, Table 4.

^b See footnote b, Table 2.

^c Percentages were calculated from the total number of strains and those transferring resistance. For *E. coli*: transferred resistance with single antibiotics, 43.3%; transferred resistance with multiple antibiotics, 56.9%. For *E. intermedia*: transferred resistance with single antibiotics, 16.6%; transferred resistance with multiple antibiotics, 57.1%.

^d Includes *Citrobacter*, *Enterobacter*, and *Klebsiella*.

TABLE 6. *WI-A2 lac⁻ recombinants which exhibited only partial resistance of the donor E. coli*

Resistance pattern of donor ^a	Resistance patterns of recombinants
Ds, Te	Ds (8) ^b
Am, Ds, Te	Am (2); Ds (3); Am, Ds (1); Am, Te (3); Ds, Te (4)
Am, C, Ds, Te	Am, Te (1); Am, C, Ds (2); C, Ds, Te (2)
Am, Cf, Ds, Te	Am, Te (1); Ds, Te (2); Am, Cf, Te (2); Am, Ds, Te (2)
Other patterns	C (1); Ds (1); Te (2); Am, Ds (1); C, Ds (1); Ds, K (2); Ds, Te (1); K, Te (1); C, Ds, Te (3); Ds, K, Te (3); Am, C, Ds, Te (1); Am, Ds, K, Te (1)

^a These donors exhibited a high degree of partial-pattern transfer (Tables 2 and 4). For abbreviations, see footnote *b*, Table 4.

^b Number of recombinants showing pattern.

TABLE 7. *Incidence of R factors identified in this study*

Resistance pattern	No.	Resistance pattern	No.
Am ^a	2	C, Ds	1
Am, Ds	2	C, Te	2
Am, Te	6	C, Ds, Te	28
Am, C, Ds	2	C, Ds, K, Te	3
Am, Cf, Te	2	Ds	12
Am, Ds, Te	19	Ds, K	11
Am, C, Ds, Te	5	Ds, Te	25
Am, Cf, Ds, Te	1	Ds, K, Te	5
Am, Ds, K, Te	1	K, Te	1
		Te	2

^a For abbreviations, see footnote *b*, Table 4.

the resistance patterns of recombinants emanating from crosses with these donors are listed in Table 6. When partial transfer occurred, resistance to ampicillin, chloramphenicol, streptomycin, and tetracycline was transferred most frequently, whereas resistance to cephalothin and kanamycin occurred less frequently. The single isolate found to be resistant to nalidixic acid (Table 4) did not transfer this resistance to the recipient.

Among the 130 multiply resistant strains which transferred resistance in whole or in part, a total of 19 different R factors were identified on the basis of the resistance patterns of recombinant colonies which grew under selective conditions from the mixed growth of various donors and

drug-sensitive recipient. The resistance patterns of these R factors and the number of times each R factor was identified are shown in Table 7. Three patterns of resistance comprised 56% of the R factors identified: the patterns chloramphenicol-dihydrostreptomycin-tetracycline, dihydrostreptomycin-tetracycline, and ampicillin-dihydrostreptomycin-tetracycline accounted for 22, 19, and 15%, respectively, of all R factors identified. The patterns dihydrostreptomycin and dihydrostreptomycin-kanamycin each accounted for 9% of the R factors identified, whereas no other R factor pattern observed accounted for more than 4% of the total R factors identified. It is of interest to note that resistance to streptomycin or tetracycline occurred in 92 and 77%, respectively, of the R factors identified, whereas streptomycin and tetracycline occurred together in 68% of the R factors.

DISCUSSION

The results of this investigation indicate that approximately 1% of the lactose-fermenting bacteria found in raw and treated sewage are multiply resistant to antibiotics commonly used for the treatment of bacterial infections in man and animals. The numbers of resistant bacteria were found to be similar in both raw and treated sewage. Our results indicate further that multiple resistance is determined by transmissible R factors in at least 50% of the strains picked at random from the various selective media used to determine the incidence of drug resistance in the sewage samples examined. In our judgement, the 50% incidence of infectious drug resistance among these strains most probably represents a minimum estimate. For example, the efficiency of transfer between donors and a particular recipient is at best about 10^{-1} to 10^{-2} per donor cell in mixed cultures under optimal laboratory conditions (10); this frequency is markedly reduced in instances in which the donor liberates bacteriophage or bacteriocins to which the recipient strain is sensitive. Anderson (1) demonstrated that the ability to transfer is not always an integral function of episomes carrying resistance markers. In our study, resistant strains which failed to transfer antibiotic resistance by mixed growth with a sensitive recipient in broth were not examined further.

Although this investigation did not distinguish between strains of human and animal origin, it is reasonable to assume that most of the resistant strains examined were of human origin. The five sewage treatment plants sampled are among seven such plants that serve an urban population

of approximately 1.2 million. Estimates of the incidence of resistant coliforms in the stools of presumably healthy individuals appear to vary widely from one report to another (2, 4, 8). This variation can be attributed to variations in the selective techniques utilized by different investigators. In England, Datta (2), utilizing techniques that permitted the detection of coliforms resistant to each of nine different antibiotics, found that 70% of newly admitted hospital patients carried resistant fecal bacteria before antibiotic therapy. At the other extreme, Gardner and Smith (4) reported the incidence of drug resistance among fecal bacteria in newly admitted hospital patients to be approximately 4%; the selective technique employed by these workers permitted the detection of only those bacteria doubly resistant to kanamycin and tetracycline. When appropriate selective techniques are employed, it appears that a significant number of presumably healthy people carry antibiotic-resistant coliforms in their intestinal tract.

The patterns of resistance exhibited by the R factors identified in this study appear to be a reflection of both clinical and nonclinical antibiotic usage. Streptomycin and tetracycline are commonly used in animal feeds (9) and are widely used clinically. Streptomycin resistance and tetracycline resistance were found in 92 and 77% of the R factors identified, respectively; they occurred together in 68% of the R factors. Resistance to chloramphenicol, which is not as widely used, was found in only 30% of the R factors, whereas resistance to gentamicin, which has only recently become available for clinical use, was not detected. Two of the R factors identified (Table 7), dihydrostreptomycin-tetracycline (19%) and ampicillin-dihydrostreptomycin-tetracycline (15%), are also frequently encountered in *E. coli* isolated from clinical materials examined in Birmingham, Alabama, hospitals (6). The R factor most frequently detected in this study, chloramphenicol-dihydrostreptomycin-tetracycline (22% of the R factors identified), would not have been seen in the absence of chloramphenicol in the selective media used. Thus, although chloramphenicol-resistant organisms were found to exhibit a high degree of transfer, it must be

emphasized that these organisms were found in numbers 10- to 100-fold lower than streptomycin- or tetracycline-resistant organisms (Tables 1 and 3).

The conclusions to be drawn from this investigation are that multiply antibiotic-resistant coliforms occur in significant numbers in both raw and treated sewage and that in at least 50% of these bacteria resistance is determined by transmissible R factors. Assuming that most of the strains examined were of human origin, the R factors identified by their patterns of resistance and the frequency of specific R factors may reflect the level of infectious drug resistance existing in the intestinal flora of the general population at any given time. Routine surveillance of sewage at periodic intervals for the detection and characterization of prevailing R factors may serve as a means of detecting significant changes in the resistance patterns of prevailing R factors and of detecting changes in the frequency of specific R factors to be found in the general population.

ACKNOWLEDGMENTS

We thank Paige Convey for excellent technical assistance.

A. B. S. was supported by general research support grant FRO-5349-08 awarded to the University of Alabama Medical School by the National Institutes of Health.

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