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Infectious Drug Resistance Among Clinically Isolated *Escherichia coli*

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Of 398 strains of clinically isolated *Escherichia coli* from three Birmingham, Alabama, hospitals, 38% were found to be resistant to one or more drugs tested. Fifty-seven per cent of the resistant strains transferred all or a part of their resistance pattern to sensitive cells during mixed cultivation. Of the 152 resistant strains, 29.1% were singly resistant, and 70.5% were resistant to more than one drug. Of the multiply resistant strains, 61% transferred all or a part of their pattern. Strains isolated from Veterans Hospital patients demonstrated higher percentages of resistance than strains isolated from Children's Hospital patients. An extremely low incidence of infective drug resistance was noted among *E. coli* isolated from the stools of healthy hospital employees.

Japanese workers first reported that resistance to several drugs can be transferred simultaneously from one bacterium to another during cell-to-cell contact (9). This type of drug resistance is called transferable or infectious drug resistance because it can spread rapidly throughout a sensitive population of cells. Infectious drug resistance is mediated by R factors which can infect all members of the *Enterobacteriaceae* (3) and which are now recognized as prevalent all over the world (2, 5, 8).

This study was done in an effort to assess the incidence of infectious drug resistance among strains of *Escherichia coli* isolated from infected patients in three Birmingham, Alabama, hospitals. Healthy hospital employees were also used as sources of *E. coli* in an attempt to define the incidence of infectively resistant organisms in the normal population.

MATERIALS AND METHODS

Bacterial strains. A total of 398 strains of *E. coli* were isolated from inpatients of Veterans, Children's, and University Hospitals by the respective clinical laboratories. The clinical material or area used for primary isolation included feces, urine, wounds, abcesses, throats, and blood. Patients may or may not have been receiving antibiotic therapy at the time of bacterial isolation. Forty-five *E. coli* strains from the stools of presumably healthy Veterans and University Hospital employees were also isolated by the respective clinical laboratories as a part of routine testing for intestinal pathogen carriers. All strains were assayed for drug resistance, and those found to be resistant to one or more drugs were used in subsequent mating experiments.

Media. Antibiotic Medium No. 3 (Difco) was used as the general supportive medium for assay of drug resistance. To prepare selective media for mating experiments, we added 250 μg of sodium azide per ml and 2% Difco agar to the Antibiotic Medium No. 3 along with either of four drugs (10 μg/ml each): chloramphenicol, tetracycline, ampicillin, or streptomycin. Selective media for assay of recombinant drug resistance were made by adding 1,000 μg of streptomycin per ml along with either 10 μg of tetracycline or chloramphenicol per ml to the antibiotic assay medium.

Drugs. Chloramphenicol was obtained as a standard preparation, compliments of Parke Davis & Co. Pharmaceutical preparations of dihydrostreptomycin and tetracycline hydrochloride, provided by Chas. Pfizer & Co., Inc., New York, N.Y., and ampicillin, provided by Ayerst Laboratories, New York, N.Y., were diluted volumetrically in sterile water. Chloramphenicol standard solutions were sterilized by membrane filtration (Millipore Corp., Bedford, Mass.) All drugs, except ampicillin, were stored by freezing.

Antibiotic sensitivity testing. Drug resistance patterns of all bacterial strains were determined by spreading 0.1 ml of an overnight broth culture of bacteria on drug-free antibiotic media agar plates. Dispens-O-Discs (Difco) were dispensed onto the surface of the plates. After incubation at 37 C for 24 hr, plates were read. Areas of inhibited growth surrounding discs were interpreted as sensitivity to the drugs. The following drugs, at 10 μg/ml, were used to determine patterns of resistance: tetracycline, ampicillin, streptomycin, chloramphenicol, and kanamycin.

Transfer of drug resistance. Clinically isolated *E. coli* strains resistant to one or more drugs were used as donors of resistance. The drug-senstivie recipient, WI-A2, was a sodium azide-resistant mutant of WI,

originally donated by D. Smith (5). WI-A2 was isolated by spreading large numbers of WI cells on solid medium containing 250 μg of sodium azide per ml. Mating procedures were carried out by mixing 1 ml of an overnight broth culture of donor and recipient in 8 ml of fresh broth and incubating without agitation overnight at 37 C. After 24 hr, 0.1 ml of mixture was spread with sterile glass rods on appropriate selective media. Growth of donor bacteria was prevented by sodium azide, whereas growth of recipient bacteria was prevented by either of the four drugs. Only drug-resistant recombinant bacteria were selected. After incubation of media for 24 hr, small, drug-resistant, recombinant colonies which had appeared were purified by picking into sodium azide broth. Drug-resistance patterns were then determined as described previously.

To test the transferability of recombinant drug resistance, it was necessary to employ WI-S, a chromosomal streptomycin-resistant mutant of WI, as the recipient. WI-S was isolated by spreading large numbers of WI cells on selective medium containing 1,000 μg of streptomycin per ml. Six recombinants were selected at random as representative of the group. Mating conditions were as described above. Selective conditions were made on the assumption that streptomycin-resistant recombinants were of an episomal nature and therefore only resistant to low levels of streptomycin. Chromosomal mutant WI-S was resistant to high levels of streptomycin but sensitive to all other drugs.

RESULTS

Drug resistance and transferability. A total of 398 strains of clinically isolated *E. coli* was obtained from three Birmingham, Alabama, hospitals, and patterns of resistance to five drugs were determined. It was found that 246, or 61.9%, of the strains were completely sensitive, whereas 152, or 38.1%, were resistant to one or more drugs. The data presented in Table 1 show that, of the 152 drug-resistant strains, 45, or 29.6%, were resistant to a single drug and 107, or 70.5%, were multiply resistant.

When tested for transfer of drug resistance, 57.6% of the resistant strains transferred all or a part of their resistance patterns, whereas in 42.4% of the strains no transfer was observed. Only 23.1% of the singly resistant strains transferred their resistance, whereas 61.6% of the multiply resistant strains did show transfer of drug resistance. One strain, singly resistant to kanamycin, was not tested for transfer.

Single drug resistance and transferability. The incidence and transfer of resistance to each separate drug was examined (Table 2). A high percentage of resistance was noted for ampicillin, tetracycline, and streptomycin, being 50.0, 67.1, and 75.6%, respectively. On the other hand, the incidence of kanamycin and chloramphenicol resistance was much lower, (19.8 and 26.3%, respectively). Transfer of ampicillin resistance was the lowest among the five drugs tested, being 42%. Transfer of chloramphenicol and kanamycin resistance was considerably higher, 72.5 and 76.7%, respectively. Tetracycline resistance was transferred in 57.0% of the tetracycline-resistant strains, whereas streptomycin resistance was transferred among 66.0% of the streptomycin-resistant strains.

Resistance patterns and transferability. Seventeen different drug resistance patterns were found among the 152 drug-resistant *E. coli* strains (Table 3). Single drug resistance was found to be fairly common to tetracycline, streptomycin, and ampicillin, whereas only one strain singly resistant to kanamycin and no strains singly resistant to chloramphenicol were isolated. Transfer of single drug resistance in the case of streptomycin resistance was relatively high, 69.3%, whereas that

TABLE 1. *Infective resistance of 152 strains of clinically isolated Escherichia coli*

Strains	No.	Per cent
Examined for drug resistance...	398	100.0
Multiply resistant.....	107/152	70.5
Singly resistant.....	45/152	29.5
Tested for transfer of resistance.	151[a]	100.0
Transferred all or part of resistance pattern.....	87/151	57.6
No resistance transfer.....	64/151	42.4
Multiply resistant strains transferring resistance.....	67/107	61.6
Singly resistant strains transferring resistance.....	19/44[a]	23.1

[a] One strain, singly resistant to kanamycin, was not tested for transferability.

TABLE 2. *Incidence of infective single drug resistance from multiply and singly resistant Escherichia coli*

Drug[a]	Incidence		Transfer	
	No. of strains resistant	Per cent	No. of strains transferring resistance/ no. of resistant strains	Per cent
Am...	75	50.0	32/75	42.6
Te....	102	67.1	58/102	57.0
C....	40	26.3	29/40	72.5
S.....	115	75.6	76/115	66.0
K....	31	19.8	23/31	74.3

[a] Abbreviations: Am, ampicillin; Te, tetracycline; C, chloramphenicol; S, streptomycin; and K, kanamycin.

TABLE 3. *Incidence and transferability of drug-resistance patterns among clinically isolated strains of Escherichia coli*

Pattern	Incidence of pattern		Transfer of pattern			
	No. of strains	Per cent	No. of strains transferring all or part of pattern/no. of strains showing pattern	Per cent	No. of strains transferring only part of pattern/no. of strains showing pattern	Per cent
Te[a]	21	13.6	10/21	48.1	—[b]	—
S	13	8.5	9/13	69.3	—	—
Am	10	6.6	0/10	00.0	—	—
K	1	0.6	—	—	—	—
S-Te	20	13.2	11/20	55.0	1/20	5.0
Am-S	15	9.9	6/15	46.0	0/15	0.0
Am-Te	5	3.3	0/5	0.0	1/5	20.0
C-S	1	0.6	0/1	0.0	0/1	0.0
K-S	2	1.3	1/2	50.0	0/2	0.0
Am-S-Te	24	15.8	17/24	71.0	6/24	25.0
Am-C-S	1	0.6	1/1	100.0	0/1	0.0
Am-K-S	1	0.6	1/1	100.0	0/1	0.0
C-S-Te	7	4.6	4/7	57.1	0/7	0.0
Am-C-S-Te	4	2.6	4/4	100.0	3/4	75.0
C-K-S-Te	11	7.3	9/11	81.9	1/11	9.1
Am-C-K-S	5	3.3	5/5	100.0	0/5	0.0
Am-C-K-S-Te	11	7.3	8/11	72.7	6/11	54.6

[a] *See* footnote *a*, Table 2.
[b] Not tested.

of tetracycline was 48.0%. No transfer of single ampicillin resistance was observed.

The incidence of certain patterns seemed relatively high enough to warrant examination. Tetracycline and streptomycin resistance occurred together in 13.2% of the resistant strains and was transferred, as a whole, among 55% of the strains exhibiting this pattern. Ampicillin and streptomycin resistance occurred in 9.9% of the strains and was transferred, as a whole, with 46% frequency. Ampicillin, streptomycin, and tetracycline resistance was the most frequently encountered pattern, occurring among 15.8% of the resistant strains. The transferability of this pattern was also significantly high, 71%, but 25% of the strains with this pattern transferred only part of the drug resistance. Ampicillin resistance failed to be transferred from four of the strains, whereas tetracycline resistance was not transferred from two strains.

All other patterns occurred less frequently, with transferability above 50% in most cases. Two other patterns showed high degrees of partial transfer among the recombinants: ampicillin, streptomycin, tetracycline, chloramphenicol; and ampicillin, streptomycin, tetracycline, kanamycin, chloramphenicol. Seventy-five per cent of the strains showing ampicillin, streptomycin, tetracycline, chloramphenicol resistance showed partial transfer, whereas 54.6% of the strains with ampicillin, streptomycin, tetracycline, kanamycin, chloramphenicol resistance partially transferred resistance markers.

Incidence of partial transfer. Table 4 gives a closer examination of the four drug-resistance patterns showing the highest degrees of partial transfer. Several recombinant colonies from each mating were picked and examined for drug resistance. Ampicillin and tetracycline resistance was found to be absent most frequently in recombinant colonies derived from matings with donors exhibiting ampicillin or tetracycline, or both, in their resistance patterns. Of all recombinants tested, 80% did not exhibit ampicillin resistance, although the donor strains had been ampicillin resistant; similarly, tetracycline resistance was absent in 71.5% of the recombinants. Streptomycin, chloramphenicol, and kanamycin resistance was absent much less frequently, 37.5, 30.8, and 34.8%, respectively.

Comparison of resistance and transfer among three hospitals. It was of interest to compare the incidence of infectious drug resistance among the three hospitals from which the strains were obtained (Table 5). A higher percentage, 62.4%, of resistance was noted among *E. coli* strains isolated from Veterans Administration Hospital than from University Hospital or Children's

TABLE 4. *WI-A2 recombinants which exhibited only a part of the original resistance pattern of the donor Escherichia coli*

Resistance pattern of donor[a]	No. of recombinants not showing resistance/no. of recombinant colonies tested				
	Am	Te	S	C	K
Am-Te	4/4	0	0	0	0
Am-S-Te	28/31	10/13	2/5	2/10	2/10
Am-C-S-Te	8/12	6/6	1/3	0	0
Am-C-K-S-Te	15/22	4/9	0	6/16	6/13
Total	55/69	20/28	3/8	8/26	8/23
Per cent	80.0	71.5	37.5	30.8	34.8

[a] These donors exhibited a high degree of partial pattern transfer (Table 3). For abbreviations, *see* footnote *a*, Table 2.

TABLE 5. *Comparison of drug resistance and transferability of clinically isolated strains of Escherichia coli among three Birmingham hospitals*

Strains	University Hospital		Veterans Hospital		Children's Hospital	
	No.	Per cent	No.	Per cent	No.	Per cent
Examined	226	100	77	100	95	100
Resistant	79	35.0	48	62.4	25	26.3
Sensitive	147	65.0	29	37.6	70	73.7
Transferring all or part of resistance	42[a]	53.2	30[b]	62.5	15[c]	60.0

[a] Of 78 resistant strains; one strain not tested for transfer.
[b] Of 48 resistant strains.
[c] Of 25 resistant strains.

Hospital. The lowest incidence of drug resistance, 26.3%, was noted from young patients in Children's Hospital. However, the incidence of infectious drug resistance proved to be approximately the same from all three hospitals: University, 53.2%; Veterans Administration, 62.5%; and Children's, 60.0%.

Incidence of drug resistance among healthy populations. It has been found that patients undergoing antibiotic therapy often excrete *E. coli* capable of transferring drug resistance (8). Consequently, it was of interest to determine whether healthy hospital employees were carriers of *E. coli* strains exhibiting infectious drug resistance. Only 2 of the 45 strains of *E. coli* were drug resistant (Table 6). However, both strains transferred their entire patterns of resistance which were (i) streptomycin and (ii) ampicillin and streptomycin.

Transferability of recombinant resistance. Episomal drug-resistant recombinants should be capable of transferring their drug resistance markers to sensitive recipients in the same manner by which they became resistant; i.e., conjugation (8). Transferability of drug resistance of six randomly selected recombinants was tested. In all cases, with a variety of patterns, transfer of drug resistance did occur from recombinant to WI-S.

TABLE 6. *Incidence of drug resistance among strains of Escherichia coli*

Strains	No.	Per cent
Examined	45	100.0
Isolated from:		
Veterans Hospital	7	15.5
University Hospital	38	84.5
Completely sensitive	43	96.0
Resistant	2	4.0

DISCUSSION

The results of this survey indicate that infectious drug resistance is probably the most common form of resistance among the pathogenic *E. coli* strains isolated from the three Birmingham, Alabama, hospitals surveyed. Nontransferable resistance was also found to be significant, but to a somewhat lesser extent, among these strains.

The fact that most of the strains were sensitive to the drugs tested is clinically encouraging. These results are similar to evidence reported by H. Smith (6), who found 42.9% resistance to one or more drugs among 70 clinically isolated *E. coli* strains from infants in Britain. He also found that the incidence of single drug resistance among diseased human beings was comparable to that presented in Table 2. However, in the same study, Smith found that 19 of 21 strains isolated transferred drug resistance. This figure is considerably higher than that found in this study. Similar high incidences of infective resistance have been reported in Japan by Mitsuhashi et al. (4). Among 160 *E. coli* strains isolated from inpatients in Japan, 84.2% were drug resistant and 66.7% were multiply resistant. Of the resistant strains, 84% transferred drug resistance.

It has been observed that patients initially excreting sensitive bacteria often excrete multiply resistant bacteria after therapy with a single drug (8). This phenomenon is probably responsible for a high incidence of multiply resistant strains as compared to singly resistant strains. In further support of this conclusion is the fact that transfer was observed in a greater per cent of the multiply resistant strains than in the singly resistant strains.

Resistance to ampicillin, tetracycline, and streptomycin, although the most frequently encountered, was transferred less often as compared to chloramphenicol and kanamycin resistance. These data infer that, although chloramphenicol and kanamycin resistance occurred less often, these resistances are more apt to be of an episomal nature among the *E. coli* strains isolated from this area than is tetracycline, streptomycin, or ampicillin resistance.

A high incidence of certain patterns of resistance involving tetracycline, streptomycin, and ampicillin was observed in Table 3. The patterns are believed to be a reflection of drugs frequently used in clinical therapy; unfortunately, a comparison of this type cannot be made in this study. It is noteworthy that transfer was observed in over half the strains with the frequently encountered resistance patterns in question. Also of interest with respect to transfer is the high percentage of transfer among the strains with patterns involving three or more drugs as compared to those with one or two resistance genes. The probability of nontransferable drug resistance to one or two drugs arising through chromosomal mutation is much greater than that for chromosomal mutation to multiple drugs, and could account for the observed differences in per cent transfer between singly and multiply resistant strains.

The complete lack of transfer of single ampicillin resistance and the lack of transfer of ampicillin resistance when it occurred as part of the pattern were noted in Tables 3 and 4. Similar results were reported previously (6, 7). The ampicillin resistance of these strains was probably chromosomal in nature, since resistant mutants of this type are easily obtained in the laboratory under selective pressures. Failure to transfer other markers, such as streptomycin and tetracycline, was also probably due to similar chromosomal mutations. Other possible explanations are: (i) segregation of resistance genes of different episomes, or (ii) the presence of a resistance gene on a defective transfer gene which was incapable of mediating transfer (1, 8).

A striking difference was noted when the percentages of sensitive *E. coli* strains isolated were compared among the three Birmingham hospitals. The differences in sensitivity might be related to present and previous antibiotic exposure of organisms. It is suggested that inpatients at Veterans Hospital, from whom the highest per cent of resistant organisms were isolated, have had more extensive exposure to various antibiotics than the younger patients at Children's Hospital, from whom the greatest per cent of sensitive *E. coli* strains were isolated. In addition, the patients at Veterans Hospital have had a greater opportunity, during their longer lifetimes, to come in contact with infectively resistant bacteria; lastly, a large majority of the patients at Veterans Hospital are suffering from chronic diseases for which they have received prolonged therapy with a variety of drugs. Strains isolated from University Hospital patients, who were of various ages, demonstrated a per cent of sensitivity intermediate between the other two values.

The incidence of healthy carriers of infectively resistant *E. coli* has been reported to be high in Britain (7). In sharp contrast are the results presented in this study. We observed a very low incidence of drug resistance among *E. coli* isolated from presumably normal hospital employees in Birmingham (Table 6). The healthy individuals surveyed came in contact, either directly or indirectly, with patients harboring infectively resistant *E. coli*. However, they did not become carriers of these strains to any great extent. This evidence is contrary to a British report (7), which suggested the possibility of cross-infection of normal individuals caring for "intensively reared" calves which were found to excrete infectively resistant *E. coli*.

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LITERATURE CITED

1. Anderson, E., and M. J. Lewis. 1965. Characterization of a transfer factor associated with drug resistance in *Salmonella typhimurium*. Nature **208**:843–849.
2. Datta, N. 1965. Infectious drug resistance. Brit. Med. Bull. **21**:254–259.
3. Harada, K., M. Suzuki, M. Kameda, and S. Mitsuhashi. 1960. On drug-resistance of enteric bacteria. 2. Transmission of drug-resistance among Enterobacteriaceae. Japan. J. Exptl. Med. **20**:289–299.
4. Mitsuhashi, S., H. Hashimoto, R. Egawa, T. Tanaka, and Y. Nagai. 1967. Drug resistance of enteric bacteria. IX. Distribution of R factors in gram-negative bacteria from clinical sources. J. Bacteriol. **93**:1242–1245.
5. Smith, D., and S. Armour. 1966. Transferable R factors in enteric bacteria causing infection of the genitourinary tract. Lancet **2**:15–18.
6. Smith, H. 1966. The incidence of infective drug resistance in strains of *Escherichia coli* isolated from diseased human beings and domestic animals. J. Hyg. **64**:465–474.
7. Smith, H., and S. Halls. 1966. Observations on infective drug resistance in Britain. Brit. Med. J. **1**:266–269.
8. Watanabe, T. 1963. Infective heredity of multiple drug resistance in bacteria. Bacteriol. Rev. **27**: 87–115.
9. Watanabe, T., and T. Fukasawa. 1960. Resistance transfer factor episome in Enterobacteriaceae. Biochem. Biophys. Res. Commun. **3**:660–665.