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Effect of Antibiotic Treatment on the Incidence of Infectious Drug Resistance Among Intestinal Lactose-Fermenting Bacteria Isolated from Burn Patients

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Antibiotic-resistant lactose-fermenting bacteria were recovered from the feces of 20 of 25 burn patients studied. Of the *Escherichia coli* isolated from patients receiving antibiotic treatment, 81.5% were shown to be infectiously resistant; only 32% of the *E. coli* recovered from patients not receiving antibiotics were shown to be harboring R factors. Three resistance patterns, ampicillin-chloramphenicol-streptomycin-kanamycin, ampicillin-tetracycline, and ampicillin-streptomycin, accounted for 25.2, 20.3, and 15.4%, respectively, of the total R factors identified.

The demonstration of infectious drug resistance among the *Enterobacteriaceae* and other unrelated gram-negative bacteria (7) has led to renewed interest in the incidence of antibiotic resistance among the gram-negative bacteria. Gill (4) suggested that antibiotic usage is the major selective force favoring the emergence of drug-resistant bacteria. Anderson (1) demonstrated that infectious drug resistance increased with antibiotic usage among livestock. Datta (2) recently suggested that the majority of all antibiotic resistant, clinically isolated gram-negative bacteria harbor R factors. There is little information currently available on the effect of antibiotic treatment on the incidence of infectious drug resistance in man.

The purpose of this study was to assess the effect of antibiotic treatment on the incidence of R factors among lactose-fermenting bacteria isolated from man. Burn patients were selected as an ideal group for study since it may be assumed that these individuals prior to admission to the hospital are representative of the normal presumably healthy population. In such patients, it should be possible to gain an insight into the effect of antibiotic treatment on infectious drug resistance among the bowel bacteria by collecting fecal specimens both before and after drug treatment and analyzing for the presence of R factors in the drug-resistant bacteria isolated. The data presented here represent the result of an initial investigation.

MATERIALS AND METHODS

Fecal specimens. Fecal specimens were obtained from burn patients at the University Hospital, Birmingham, Ala., upon admission and at weekly intervals thereafter. A total of 47 specimens from 25 patients were studied. Patients' charts were reviewed at routine intervals to determine the antibiotic treatment, if any, being administered.

Isolation of resistant bacteria. A portion of fecal material (about the size of a pea) was emulsified in 0.5 ml of 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 and adjusted to *p*H 7.1 with hydrochloric acid]. A portion was then spread over the entire surface of a MacConkey Agar (BBL) plate with a sterile cotton-tipped swab. Sensi-Discs (BBL) were then dispensed onto the surface of the seeded plate. The following antibiotic discs were used: ampicillin, 10 μg; chloramphenicol, 5 μg; cephalothin, 30 μg; dihydrostreptomycin, 10 μg; gentamicin, 10 μg; kanamycin, 30 μg; nalidixic acid, 5 μg; sulfachloropyridazine, 250 μg; and tetracycline, 5 μg. After incubation at 37 C for 18 to 24 hr, a portion of the resistant growth from around five randomly selected discs was picked, suspended in TM buffer, and streaked onto a fresh MacConkey Agar plate. After overnight incubation, a single well-isolated lactose-fermenting colony was inoculated to a Kligler Iron Agar (BBL) slant which served as a stock culture after incubation at 37 C. Each isolate was subsequently identified by the methods outlined by Edwards and Ewing (3).

Antibiotic-susceptibility testing. Drug-resistance patterns of all isolates were determined by spreading a portion of a 3- to 4-hr broth culture of the organism

TABLE 1. *Incidence and transferability of resistance patterns among Escherichia coli isolated from patients receiving antibiotic treatment*

Resistance pattern[a]	No.	Percent of total	Transferred resistance
Am, Ds	4	3.7	1
Ds, Te	1	0.9	(1)[b]
Am, Cf, Ds	7	6.5	(4)
Am, Ds, K	5	4.6	(1)
Am, Ds, Te	36	33.3	1(31)
Ds, K, Te	3	2.8	(3)
Am, C, Ds, K	11	10.2	10
Am, C, Ds, Te	1	0.9	(1)
Am, Cf, Ds, Te	4	3.7	(3)
Am, Cf, Ds, K	2	1.9	(2)
Am, Ds, K, Te	2	3.7	(2)
Am, C, Ds, K, Na	1	0.9	(1)
Am, C, Ds, K, Te	19	17.6	3(14)
Am, C, Cf, Ds, K	10	9.3	1(8)
Am, Cf, Ds, K, Te	1	0.9	0
Am, C, Cf, Ds, K, Te	1	0.9	(1)
Total[c]	108		16(72)

[a] Abbreviations: Am, ampicillin; C, chloramphenicol; Cf, cephalothin; Ds, dihydrostreptomycin; K, kanamycin; Na, nalidixic acid; Sl, sulfachloropyridazine; and Te, tetracycline.

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

[c] Of the 108 isolates, 88 or 81.5% transferred all or part of their resistance to a sensitive recipient.

to be tested onto Brain Heart Infusion Agar (Difco). Sensi-Discs of the same type and concentration as described above were dispensed onto the surface of the seeded plates, and the resistance pattern was determined after incubation at 37 C for 18 to 24 hr.

Transfer of antibiotic resistance. Lactose-fermenting isolates found to be resistant to one or more antibiotics were used as prospective donors of resistance to an F^- derivative of *Escherichia coli* K-12. The lactose-negative recipient, designated WI-A2 (*lac*$^-$), is completely susceptible to antibiotics but resistant to 250 μg of sodium azide per ml (5, 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 Brain Heart Infusion Broth (Difco). The mixtures were incubated for 18 to 24 hr as a stationary culture. A swabful of each mixture was smeared onto MacConkey Agar plates containing a single appropriate antibiotic and 250 μg of sodium azide per ml. In this manner, 12 mixtures could be placed on each plate. The media used were selective for antibiotic-resistant recombinants of WI-A2 (*lac*$^-$) in that 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 picked and streaked to the same selective medium to ensure pure colony isolation. The antibiotic-resistance pattern of at least one recombinant colony from each mating mixture was determined as described above to ascertain whether partial or complete transfer of resistance from donor to recipient had taken place.

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

RESULTS

Drug resistance and transferability among bacteria isolated from patients receiving antibiotics. Drug-resistant lactose-fermenting bacteria were recovered from 20 of the 25 burn patients studied. Resistant bacteria were recovered from all 11 patients who received antibiotics. The patients included in the treated group received penicillin, ampicillin, nitrofurantoin, and gentamicin with the majority receiving prophylactic doses of penicillin.

A total of 142 lactose-positive strains were isolated from the MacConkey Agar plates containing Sensi-Discs. Further characterization of these isolates revealed that 108 were *E. coli*, whereas 34 belonged to the *Klebsiella-Enterobacter* group. The drug resistance pattern of each isolate was determined by using nine different antibiotics. The drug-resistance patterns observed as well as the incidence of infectious drug resistance among the *E. coli* isolates recovered

TABLE 2. *Incidence and transferability of resistance patterns among Klebsiella-Enterobacter isolated from patients receiving antibiotic treatment*

Resistance pattern[a]	No.	Percent of total	Transferred resistance
Am	2	5.9	1
Am, Te	1	2.9	1
Am, C, Ds, Na	13	38.2	(2)[b]
Am, C, Ds, K	1	2.9	1
Am, C, K, Te	1	2.9	0
Am, C, Ds, Na, Sl	6	17.6	(1)
Am, C, Ds, K, Na	1	2.9	(1)
Am, C, Ds, K, Te	5	14.7	(2)
Am, C, Cf, Ds, K, Te	3	8.8	(1)
Am, C, Ds, Na, Sl, Te	1	2.9	0
Total[c]	34		3 (7)

[a] See footnote *a*, Table 1.

[b] See footnote *b*, Table 1.

[c] Of the 34 isolates, 10 or 29.4% transferred all or part of their resistance to a sensitive recipient.

TABLE 3. *Incidence and transferability of resistance patterns among Escherichia coli isolated from patients not receiving antibiotics*

Resistance pattern[a]	No.	Percent of total	Transferred resistance
Am	3	4.8	0
C	1	1.6	0
Am, Ds	7	11.3	(1)[b]
Am, Cf	2	3.2	0
Ds, K	3	4.8	1 (1)
Ds, Te	5	8.0	(2)
Am, C, Na	3	4.8	0
Am, Ds, Te	22	35.5	5 (2)
Ds, K, Te	1	1.6	0
Am, Ds, K, Te	3	4.8	0
Am, C, Ds, K	6	9.7	4
Am, C, Cf, K	1	1.6	1
Am, C, Ds, Te	1	1.6	0
Am, C, Cf, Ds, K	1	1.6	0
Am, C, Ds, K, Te	3	4.8	1 (2)
Total[c]	62		12 (8)

[a] See footnote *a*, Table 1.
[b] See footnote *b*, Table 1.
[c] Of the 62 isolates, 20 or 32.2% transferred all or part of their resistance to a sensitive recipient.

from the patients receiving antibiotic treatment are shown in Table 1. All of the 108 strains were resistant to two or more antibiotics. The most common resistance patterns observed included resistance to various combinations of ampicillin, chloramphenicol, cephalothin, kanamycin, streptomycin, and tetracycline. The patterns occurring most frequently were ampicillin-streptomycin-tetracycline, ampicillin-chloramphenicol-streptomycin-kanamycin-tetracycline, ampicillin-chloramphenicol-streptomycin-kanamycin, and ampicillin-chloramphenicol-cephalothin-streptomycin-kanamycin which accounted for 36 (33.3%), 19 (17.6%), 11 (10.2%), and 10 (9.3%), respectively, of all the patterns identified. The ampicillin-streptomycin-tetracycline pattern was transferred whole or in part to a sensitive recipient by 89% of the *E. coli* strains exhibiting the pattern. It was shown that 100% of the *E. coli* strains were resistant to streptomycin and 96 and 51%, respectively, of the strains were resistant to ampicillin or kanamycin. Of the 108 *E. coli* isolates, 88 or 81.5% transferred all or part of their resistance to a susceptible recipient.

Table 2 shows the incidence and transferability of resistance patterns among the 34 *Klebsiella-Enterobacter* strains recovered from patients receiving antibiotic treatment. All 34 strains were resistant to ampicillin, whereas 31 (91%), 30 (87%), and 21 (62%), respectively, were resistant to chloramphenicol, streptomycin, or nalidixic acid. Of the 34 strains studied, 13 or 38% were multiply resistant to ampicillin, chloramphenicol, streptomycin, and nalidixic acid. In contrast to the 81.5% of *E. coli* strains shown to be infectiously resistant, only 29.4% of the *Klebsiella-Enterobacter* isolates harbored R factors.

Drug resistance and transferability among bacteria isolated from patients not receiving antibiotics. Of the 18 patients studied who were not receiving antibiotic treatment, 13 were found to have drug-resistant, lactose-positive bacteria in their fecal flora. Seventy-seven strains were recovered from the primary isolation medium. After purification and further characterization, 62 of the isolates were identified as *E. coli* and 15 as belonging to the *Klebsiella-Enterobacter* group. The drug-resistance patterns of all 77 strains were determined, and each strain was grown in mixed culture with the drug-susceptible recipient to assay for resistance transfer. The incidence and transferability of resistance patterns among the *E. coli* recovered from patients not receiving antibiotics are shown in Table 3. One pattern, ampicillin-streptomycin-tetracycline, accounted for 35.5% of all the resistance patterns observed. Of the 62 *E. coli* strains studied, 52, 52, and 35 (84, 84, and 57%) were resistant to ampicillin, streptomycin, or tetracycline, respectively.

It is of interest that only 15 (24%) of the *E. coli* recovered from the untreated group were resistant to four or more antibiotics, whereas 52 (46%) from the treated group were resistant to four or more antibiotics. Furthermore, only 32% of the *E. coli* recovered from the untreated group were found to be infectiously drug resistant; 81.5% isolated from the treated group transferred resistance.

TABLE 4. *Incidence and transferability of resistance patterns among Klebsiella-Enterobacter isolated from patients not receiving antibiotics*

Resistance pattern[a]	No.	Percent of total	Transferred resistance
Am	5	33.3	0
Am, Na	1	6.7	0
Am, Te	1	6.7	0
C, Ds, Te	1	6.7	0
Am, C, Ds, Te	1	6.7	0
Am, Cf, Ds, Te	4	26.7	(3)[b]
Am, C, Ds, K, Te	2	13.3	(1)
Total[c]	15		(4)

[a] See footnote *a*, Table 1.
[b] See footnote *b*, Table 1.
[c] Of the 15 isolates, 4 or 26.6% transferred all or part of their resistance to a sensitive recipient.

TABLE 5. *Summary of incidence of R factors identified*[a]

Resistance pattern[b]	*E. coli*	*Klebsiella-Enterobacter*	Per cent of total
Am	2	1	2.4
Ds	5	0	4.1
Te	1	0	0.8
Am, Ds	16	3	15.4
Am, Te	24	1	20.3
C, K	0	1	0.8
Ds, K	4	0	3.3
Ds, Na	0	1	0.8
Am, C, Ds	1	0	0.8
Am, Ds, K	2	0	1.6
Am, Ds, Te	11	3	11.4
Am, C, Ds, K	28	3	25.2
Am, C, Cf, K	1	0	0.8
Am, C, Ds, Te	2	0	1.6
Am, C, K, Te	7	0	5.7
Am, C, Cf, Ds, K	1	0	0.8
Am, C, Ds, K, Te	4	1	4.1

[a] The isolates are from both groups of patients.
[b] See footnote *a*, Table 1.

Table 4 summarizes the results obtained with the *Klebsiella-Enterobacter* strains recovered from the untreated group. Although only 15 strains were recovered from this group, it may be seen that the isolates were less resistant than the *Klebsiella-Enterobacter* recovered from the treated group. The incidence of infectious drug resistance (26.6%), however, was not significantly different from that observed among strains recovered from the treated group (29.4%).

R factors identified. Among the 170 *E. coli* and 49 *Klebsiella-Enterobacter* isolates studied, 123 strains transferred their resistance either totally or partially. Table 5 shows the 15 different R factors identified in this study. Three resistance patterns, ampicillin-chloramphenicol-streptomycin-kanamycin, ampicillin-tetracycline, and ampicillin-streptomycin, accounted for 25.2, 20.3, and 15.4%, respectively, of the total R factors identified. Of the 123 strains bearing R factors, 93, 39, 68, 42, and 42% were resistant to ampicillin, chloramphenicol, streptomycin, kanamycin, or tetracycline, respectively.

DISCUSSION

Drug-resistant, lactose-fermenting bacteria were isolated from 20 of 25 burn patients studied. Drug-resistant *E. coli* isolated from feces of burn patients receiving antibiotic treatment showed a 2.5-fold greater incidence of infectious drug resistance when compared to the untreated group. It appeared that this response to antibiotic therapy was entirely nonspecific in that treatment with one antibiotic appeared to induce an increased rate of transfer of resistance to other antibiotics. It was found that the drug-resistance patterns of the *E. coli* recovered from patients receiving either "prophylactic" penicillin or ampicillin, nitrofurantoin, or gentamicin were essentially the same. Datta (2) also found no direct relationship between drug usage and the resistance patterns of her isolates.

These results indicate antibiotic treatment as a selective force for R factors. However, the possibility exists that infectiously resistant bacteria could have been hospital-acquired since the median hospitalization for the treated group (45 days) was three times greater than the untreated group (15 days). This appears unlikely since three patients receiving no antibiotics and hospitalized for periods equal to the treated group showed no increase in the frequency of the presence of R factors. In agreement with our results, Datta (2) found that the length of hospitalization beyond 3 weeks did not affect the number of resistant bacteria recovered from fecal specimens. However, Datta (2) demonstrated that there was a tendency to acquire drug-resistant bowel bacteria, in particular *E. coli*, during the first 3 weeks after admission. Datta did not determine whether the increase in resistant bacteria was due to the acquisition of resistance by originally sensitive strains or the replacement of the original strains with resistant ones.

Anderson (1) showed R factors to be composed of a resistance factor (*rf*) and a resistance-transfer factor (*rtf*) and suggested that these components, initially independent, may recombine to form a complete R factor after conjugation of a bacterium carrying an *rf* and one harboring an *rtf*. Anderson also found that the *rtf* transfers at a much higher rate than the complete R factor. It would then be possible for antibiotic treatment to select for drug-resistant bacteria in the bowel and thus enrich for *rf*- and R factor-containing bacteria. If, after enrichment of resistant bacteria by antibiotics, a bacterium containing an *rtf* came in contact with those bacteria containing an *rf* only, the *rtf* and *rf* could recombine to produce a complete R factor after conjugation. This could account for the increase in infectious drug resistance among patients receiving antibiotics.

The conclusions to be drawn from this investigation are that multiply antibiotic-resistant coliforms are easily recovered from the feces of persons who may or may not be receiving antibiotic treatment. *E. coli* isolated from patients

receiving antibiotics showed a 2.5-fold greater incidence of infectious drug resistance than did those recovered from patients not receiving antibiotic treatment. This response appeared to be the same regardless of the antibiotic used for treatment.

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