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# High Frequency of Antimicrobial Resistance in Human Fecal Flora

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**The frequency of resistance to seven different antimicrobial agents was examined in the aerobic gram-negative gut flora of over 600 individuals from hospitals, from laboratories where antibiotics were used, and from urban and rural communities. In a majority (62.5%) of fecal samples from people without a recent history of taking antibiotics, 10% or more of the total organisms were resistant to at least one of the antibiotics. In about 40% of the samples, resistance to more than one drug was present at this level. More than one-third of the samples contained resistant organisms comprising 50% or more of the total flora examined. Organisms with coresistance to multiple drugs were found frequently. Individuals taking antibiotics produced more samples with a higher proportion (>50%) of resistant bacteria, and these samples also had a significantly greater number of different resistance determinants. This extensive study revealed a high prevalence of resistant bacteria in the gut flora of ambulatory and hospitalized individuals whether or not they were taking antibiotics.**

There is worldwide concern about the appearance of antibiotic resistance in common pathogens of hospital- and community-acquired infections (4, 9, 20). While selective pressure caused by antibiotic usage has been linked to these findings (20), there have been little data on the natural frequency of antibiotic resistance genes in the normal nonpathogenic flora of ambulating and hospitalized individuals. Such resistant bacteria would represent a constant pool of resistance genes potentially transferable, directly or indirectly, to human pathogens. We analyzed fecal specimens from humans in the greater Boston, Mass., area for both the presence and frequency of naturally occurring aerobic gram-negative bacteria with resistance to one or more of seven different antimicrobial agents. We found that resistance was common in the gut flora and the resistant strains constituted a major fraction of the total flora in more than one-third of the samples. This density of resistance was found in samples from individuals who did not ingest antibiotics as well as from those who did.

## MATERIALS AND METHODS

**Description of sample populations.** Over a 16-month period (December 1977 through March 1979), we collected and tested 974 fecal samples from 640 hospitalized and ambulatory human donors. The population consisted of hospitalized as well as normal ambulatory people. The patient samples were obtained from four medical wards of the New England Medical Center Hospital and the clinical study unit. The ambulatory population consisted of laboratory workers and urban and rural dwellers. The laboratory donors came from eight greater Boston research and clinical laboratories; 10 samples representing seven donors were obtained from laboratory workers at the University of Georgia, Athens. The urban population was composed of first-year medical students and instructors from the Tufts University and Harvard University medical schools and family members of laboratory workers. None had contact with patients or hospitals. The rural population consisted of families residing in Sherborn, Mass., located 25 miles west of Boston. Most of the ambulatory contributors answered a questionnaire which included the following items: sex, age, communities of residence and employment, and history of antibiotic therapy within the previous 2 weeks or exposure through working with antibiotics.

The donors were predominantly adult (95.4% over age 15) with a male/female distribution of approximately 0.8:1 (Table 1). The mean age of females (45 ± 22.1 years) was slightly higher than that of males (39.9 ± 20.9 years); the mean age of the total population studied was 42.7 ± 21.7 years, with a median age of 37 years (Table 1).

In September 1987, a second smaller sampling was performed on a group of 80 second-year Tufts medical students. The age range of this group was 22 to 41 years, with a mean of 24.5 years and a median of 24 years. No member of this group reported antibiotic ingestion in the previous 6 months; none had patient contact. University guidelines were followed for obtaining human fecal samples.

**Sample collection and processing.** Fresh fecal samples from hospitalized patients were collected directly into a plastic container (Lab-Tek; Miles Scientific, Div. Miles Laboratories, Inc., Naperville, Ill.) which was refrigerated immediately and transported to the laboratory within 24 h. Each fecal mass was sampled internally by inserting a sterile swab into the center. Samples from nonhospitalized individuals were collected by the same means or by a sterile cotton-tipped applicator (Culturette; Scientific Products) inserted into the rectum or directly into fresh feces. Each Culturette was immediately crushed to release Stuart transport medium. Many individuals were sampled more than once to evaluate the stability of a given gut profile. All individuals provided their own samples and consented to the use of their excrement for this analytic study. Except when noted, all statistics were based on results derived from the first fecal specimen collected from each donor. The data on multiple sampling were treated separately.

Each swab was plated onto two plates of MacConkey agar by inoculating one quadrant of each plate. The inoculum was then diluted by two-dimensional streaking onto the remaining three quadrants. All plates were incubated overnight at 37°C. From each plate, 50 to 200 clearly isolated colonies

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TABLE 1. Study population

| Group | Total no. of individuals sampled | Sex distribution | | Age (yr)[a] | |
|---|---|---|---|---|---|
| | | Females[b] | Males[c] | Range | Mean ± SD[d] |
| Hospital patients | 298 | 160 | 127 | 12–95 | 59.7 ± 17.2 |
| Laboratory workers | 130 | 65 | 56 | 19–60 | 28.9 ± 7.5 |
| Urban dwellers | 109 | 51 | 58 | 1–64 | 26.5 ± 11.6 |
| Rural dwellers | 62 | 30 | 29 | 1–61 | 25.4 ± 17.1 |
| Other[e] | 41 | 29 | 8 | 23–63 | 33.4 ± 14.7 |

[a] Based on the following sample sizes of known age: hospital, 297; laboratory workers, 120; urban dwellers, 109; rural dwellers, 59; other, 37.
[b] Mean age (includes only those whose sex was known) ± standard deviation, 45 ± 22.1 years; range, 1 to 92 years.
[c] Mean age (includes only those whose sex was known) ± standard deviation, 39.9 ± 20.9 years; range, 1 to 95 years.
[d] The median age for the population studied was 37 years; the mean age was 42.7 ± 21.7 years.
[e] Consists of 11 hospital staff and 30 vegetarians.

were enumerated and classified as lactose fermenters and lactose nonfermenters.

The best of each pair of MacConkey plates was selected for replica plating (5) onto a series of antibiotic-containing MacConkey plates (each containing one of the following antimicrobial agents: ampicillin, 30 μg/ml; streptomycin, 30 μg/ml; tetracycline, 10 μg/ml; kanamycin, 10 μg/ml; chloramphenicol, 25 μg/ml; gentamicin, 10 μg/ml; and nalidixic acid, 30 μg/ml), followed by a plate of plain MacConkey agar. Samples from the hospital groups were also tested with cephalothin, 30 μg/ml. Samples taken in the 1987 survey were tested with the following antibiotics: ampicillin, streptomycin, tetracycline, and kanamycin. Trimethoprim at 2 μg/ml in Mueller-Hinton agar was also tested. After overnight incubation at 37°C, the plates were again tabulated by comparison with the replica-plated control plate of MacConkey agar alone. Numbers of the various colony types resistant to each drug were recorded. From control studies, the levels of antibiotic used and the size of the colonies recovered excluded the possibility of counting spontaneous chromosomal mutants.

The data obtained from each sample were converted to an alpha-numeric coding system and analyzed by computer, using the SPSS (Statistical Package for the Social Sciences) program (SPSS, Inc., Chicago, Ill.). The data were organized at two levels in the fecal sample: ≥10 and ≥50% resistant organisms. Statistical significance was determined by the chi-square test.

## RESULTS

**Frequency of antibiotic resistance among fecal specimens from four different human populations.** Gram-negative aerobic fecal bacteria from hospitalized individuals, those working in laboratories, or those living in urban or rural communities were examined for lactose (Lac) fermentation and antibiotic resistance. About two-thirds (62.7%) of the samples showed $Lac^+$ colonies only. Another 36.4% contained both $Lac^+$ and $Lac^-$ organisms; however, among these samples, the majority of colonies (>90%) were $Lac^+$. Six (<1%) had $Lac^-$ organisms only. The Lac phenotype distribution was not different between those individuals known to be taking ("on") or not taking ("off") antibiotics. Since the $Lac^-$ populations were commonly absent or comprised a minor population, we focused on $Lac^+$ organisms as representative of the general phenotype of the total fecal flora.

We compared the antibiotic resistance profiles of the $Lac^+$ fecal flora found in samples from males and females (Fig. 1). More than 30% of the fecal samples from both groups had ≥10% of organisms resistant to ampicillin ($Ap^r$), streptomycin ($Sm^r$), tetracycline ($Tc^r$), or cephalothin ($Cf^r$). Between 10 and 25% of the samples had ≥50% of the bacteria resistant to these drugs. A quarter of the samples contained ≥10% bacteria resistant to kanamycin ($Kn^r$), and 8 to 10% were predominantly resistant (≥50% level). There was much less resistance to chloramphenicol ($Cm^r$), gentamicin ($Gm^r$), or nalidixic acid ($Na^r$) (Fig. 1). No significant differences were found between males and females; therefore, the data from these two populations were combined for all subsequent analyses. No significant differences were seen among samples from different laboratory workers (data not shown), so these samples were combined for analysis.

We examined the individuals off antibiotics in the population sampled. In a majority (62.5%) of all fecal samples, 10% or more of the flora were resistant to one or more of the antibiotics tested. Over one-third (37.9%) of the samples showed resistance(s) in ≥50% of the flora (Table 2). When the people off antibiotics were subdivided into hospitalized and ambulatory groups (Table 2), it was seen that fecal samples from hospital patients harbored more $Ap^r$ strains at both frequency levels ($P < 0.01$). At the ≥50% frequency level, samples from the patients showed more resistance to kanamycin, but not significantly ($P < 0.1$). There were, however, no other significant differences in the samples from

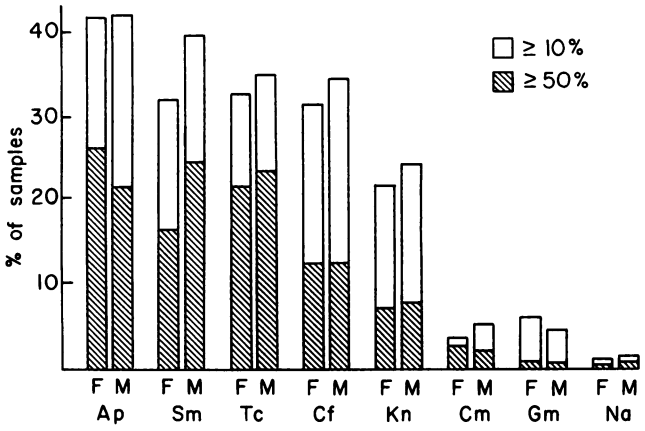


FIG. 1. Antibiotic resistance among $Lac^+$ flora in fecal samples from females (F) and males (M). The relative amount of resistant colonies (≥10 or ≥50% of $Lac^+$ bacteria on each MacConkey agar plate) in each fecal sample was recorded for each antibiotic (given on bottom line). Ap, Ampicillin; Sm, streptomycin; Tc, tetracycline; Cf, cephalothin; Kn, kanamycin; Cm, chloramphenicol; Gm, gentamicin; Na, nalidixic acid. Data for cephalothin came from the hospital group only.

TABLE 2. Frequency of antibiotic resistance in individuals not receiving antibiotics

| Population (sample size) | Resistance frequency (%)[a] | % Resistant[b] | % Resistant to given antibiotic[c] | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | Ap | Sm | Tc | Kn | Cm | Gm | Na |
| Patients (189) | ≥10 | 67.2 | 48.2[d] | 34.4 | 38.1 | 24.9 | 4.2 | 7.4 | 0.5 |
| | ≥50 | 45.0 | 27.0[e] | 21.2 | 22.8 | 7.9[f] | 1.6 | 0.5 | 0.5 |
| Nonpatients (289) | ≥10 | 59.5 | 34.9[d] | 33.6 | 30.1 | 20.4 | 3.8 | 4.5 | 0.7 |
| | ≥50 | 33.5 | 16.7[e] | 15.6 | 18.7 | 3.5[f] | 1.7 | 0.4 | 0.7 |
| Total (487)[g] | ≥10 | 62.5 | 39.6 | 33.5 | 32.8 | 22.2 | 3.9 | 5.9 | 0.6 |
| | ≥50 | 37.9 | 20.3 | 17.5 | 19.9 | 5.1 | 1.6 | 0.4 | 0.6 |

[a] Of total Lac$^+$ organisms.
[b] Samples with designated frequency of resistance to any of the seven drugs.
[c] Ap, Ampicillin; Sm, streptomycin; Tc, tetracycline; Kn, kanamycin; Cm, chloramphenicol; Gm, gentamicin; Na, nalidixic acid.
[d] $x^2 = 7.74$; $P < 0.01$.
[e] $x^2 = 6.87$; $P < 0.01$.
[f] $x^2 = 3.76$; $P < 0.10$.
[g] Includes nine hospital staff members.

the two groups off antibiotics in terms of resistance to the other five antibiotics tested.

A comparison among the individuals off antibiotics from the four subpopulations studied revealed that one-fourth or more of each group produced samples which contained Ap$^r$, Sm$^r$, and Tc$^r$ (in the same or different bacteria) at the ≥10% frequency level (Fig. 2A). Resistance to kanamycin was somewhat lower. In all populations, <10% of the samples carried Cm$^r$, Gm$^r$, and Na$^r$ bacteria at the ≥10% frequency level (Fig. 2B). There were no significant differences among the three ambulatory groups. The general trend, however, was towards lower numbers of resistant bacteria in the rural samples, and Cm$^r$ was absent from this population. In contrast to other groups, approximately 4% of rural samples had >10% Na$^r$ bacteria (Fig. 2B). This finding was due to an Na$^r$ *Escherichia coli* isolate carried by several members of a single family.

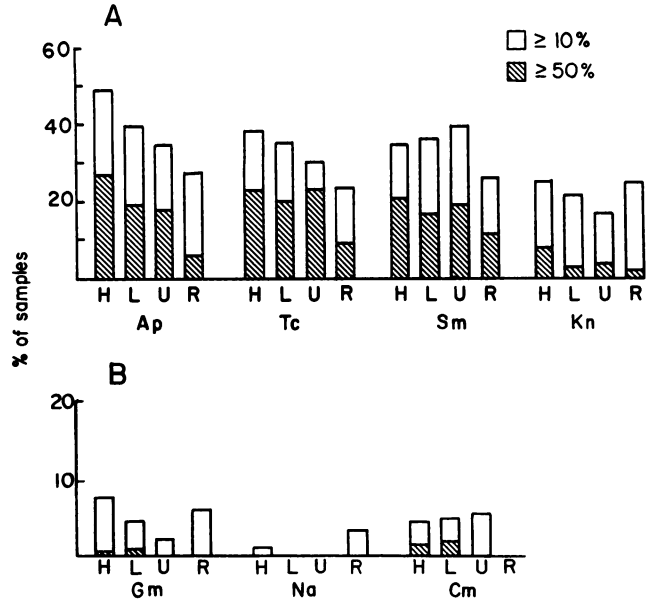


FIG. 2. Frequency of antibiotic-resistant Lac$^+$ organisms in fecal samples from four human populations not ingesting antibiotics: hospital (H), laboratory (L), urban (U), and rural (R). See legend to Fig. 1. Significant differences in ampicillin resistance were found among hospital, urban, and rural groups (see text).

Several differences appeared when the samples from each ambulatory group were compared with those from the hospitalized patients (Fig. 2A). Both the urban and the rural group samples had significantly less Ap$^r$ than the hospital group (urban: $\chi^2 = 4.36$, at the 10% level only, with $P < 0.05$; rural $\chi^2 = 6.17$ at the ≥10% level, with $P < 0.05$, and $\chi^2 = 9.0$ at the ≥50% level, with $P < 0.01$).

**Effect of antibiotic usage on frequency of resistant organisms.** Most of the antibiotic usage among people tested in this study occurred in the hospital. Fifty-three patients (of the 247 patients whose antibiotic use was known) were receiving one or more antibiotics. An additional 12 of 314 ambulatory donors (whose antibiotic use was known) had taken an antibiotic within 2 weeks prior to sampling. Given the small number of nonhospitalized individuals on antibiotics and the minimal difference found among hospitalized and nonhospitalized individuals off antibiotics (Table 2), we focused on the hospitalized group to determine the effect of antibiotic use. The only significant difference was the higher percentage of fecal samples with Ap$^r$ bacteria from the group on antibiotics (at the ≥10% frequency level, $\chi^2 = 6.7$, $P < 0.01$; at the ≥50% resistance frequency, $\chi^2 = 11.9$, $P < 0.001$). Detectable, but not significant, differences were also noted to four other antibiotics: streptomycin, tetracycline, kanamycin, and cephalothin (Fig. 3).

**Frequency of more than one antibiotic resistance determinant in fecal flora.** Fecal samples commonly contained more than one resistance determinant in the same or different organisms. Almost 40% of all samples had multiple determinants at ≥10% levels (Fig. 4). Slightly greater numbers of samples from those on antibiotics contained more than one resistance determinant (two or more drugs) at the ≥10% frequency level compared with samples from those not taking drugs (Fig. 4). This difference was significant when more than four resistances were present (23 versus 10%; $P \leq 0.01$). At the higher frequency level (≥50%), significant differences were found between the two groups in the number of samples with multiple resistance determinants (Fig. 4). The group on antibiotics had two- to threefold more samples with more than two and three resistances and ninefold more samples with more than four resistances.

A total of 364 samples were randomly chosen from among those off antibiotics for analysis of multiple resistance within the same organism (coresistance). Seventy-one percent carried detectable levels of one or more of 31 different coresistance patterns. The most frequently occurring patterns were

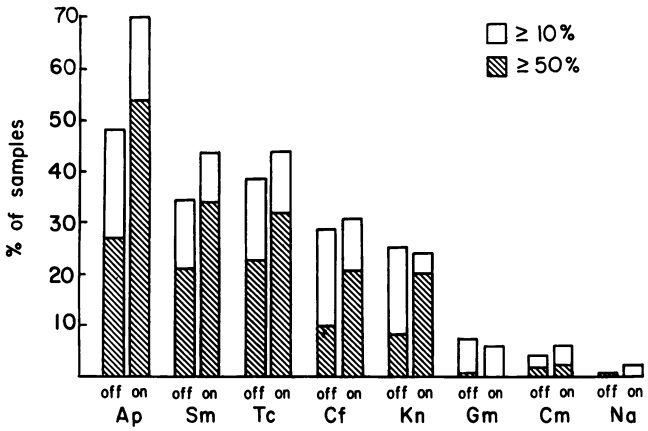


FIG. 3. Effect of antibiotic usage on antibiotic resistance frequency. Fecal samples from patients off and on antibiotics were compared for frequency of resistant bacteria at two levels. See legend to Fig. 1. Significant differences appeared in ampicillin resistance at both levels (see text).

$Sm^r$ $Tc^r$ (22.3%), $Ap^r$ $Sm^r$ $Tc^r$ (16.5%), and $Ap^r$ $Sm^r$ $Tc^r$ $Kn^r$ (14.6%). Twelve other coresistance patterns were detectable in ≥1% of positive samples (Table 3).

**Comparison of resistance between single- and multiple-sample analysis.** We compared the data generated from single samples with those generated from all samples. No statistically significant differences were found at any resistance level to any of the antibiotics (Table 4). Further examination of data from the multiple-sample group revealed that changes in resistance patterns of gut flora were extremely common and occurred frequently within a 2-week period. Some 143 pairs of resistance patterns were examined, using the first two samples obtained from each individual of the ambulatory group off antibiotics. Ninety percent of all individuals showed a gain (47.6%) and/or loss (65.7%) of one or more detectable resistances. Forty-four percent exhibited a gain or complete loss of a resistance marker at the ≥10% level.

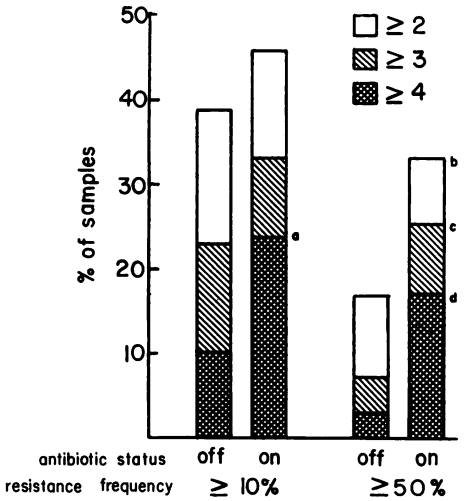


FIG. 4. Frequency of different resistance determinants (more than two, three, or four) in fecal samples from individuals on or off antibiotics. Significant values were as follows: a, $\chi^2 = 8.3$, $P < 0.01$; b, $\chi^2 = 8.1$, $P < 0.01$; c, $\chi^2 = 20.4$, $P < 0.001$; d, $\chi^2 = 26.6$, $P < 0.001$.

TABLE 3. Frequency of common coresistance patterns[a]

| Coresistance pattern | No. of samples[b] | Frequency (%)[c] |
|---|---|---|
| $Sm^r$ $Tc^r$ | 58 | 22.3 |
| $Ap^r$ $Sm^r$ $Tc^r$ | 43 | 16.5 |
| $Ap^r$ $Sm^r$ $Tc^r$ $Kn^r$ | 38 | 14.6 |
| $Sm^r$ $Tc^r$ $Kn^r$ | 17 | 6.5 |
| $Ap^r$ $Tc^r$ | 19 | 7.3 |
| $Ap^r$ $Sm^r$ | 19 | 7.3 |
| $Ap^r$ $Kn^r$ | 13 | 5.0 |
| $Sm^r$ $Kn^r$ | 13 | 5.0 |
| $Ap^r$ $Sm^r$ $Kn^r$ | 12 | 4.6 |
| $Tc^r$ $Kn^r$ | 8 | 3.1 |
| $Ap^r$ $Sm^r$ $Tc^r$ $Kn^r$ $Gm^r$ | 7 | 2.7 |
| $Ap^r$ $Sm^r$ $Tc^r$ $Kn^r$ $Cm^r$ | 5 | 1.9 |
| $Kn^r$ $Gm^r$ | 5 | 1.9 |
| $Tc^r$ $Cm^r$ | 5 | 1.9 |
| $Sm^r$ $Cm^r$ | 4 | 1.5 |

[a] Only those coresistance patterns occurring in 1% or more of the samples are presented.
[b] Number of fecal samples of 364 tested for coresistance.
[c] Frequency of coresistance pattern among 260 samples which contained coresistance.

**Repeat study of the urban group.** The urban-dwelling group of medical students sampled in the 1987 survey consisted only of those off antibiotics. In this group, 46.3% of samples showed 10% or more of the flora having $Ap^r$, $Sm^r$, $Tc^r$, $Kn^r$, or $Tm^r$ strains. Resistance(s) in ≥50% of the flora was found in 26.4% of the samples. Resistance to the individual drugs at the ≥10% level was as follows: $Ap^r$, 25%; $Sm^r$, 28.8%; $Tc^r$, 38.8%; and $Kn^r$, 15%. High-frequency resistance (≥50% of the fecal flora) was 11.3% for ampicillin, 17.5% for streptomycin, 21.3% for tetracycline, and 7.5% for kanamycin. Trimethoprim resistance (not assayed in the earlier study) was found in 7.5% of the samples from this group. Only a few samples (2.5%) bore this resistance in ≥50% of the flora. These findings were very similar to those of the urban group studied 8 to 9 years earlier.

Multiple resistance in samples from the medical students in the 1987 survey was very similar to that obtained from the off antibiotic group of the earlier study. Thirty-one percent of the samples contained multiple (more than two) resistance determinants at the ≥10% level, 17.5% bore three or more resistances, and 10% had flora with resistance to all four drugs tested in both studies. At the ≥50% level, 15% had multiple (more than two) resistances, 7.5% had more than three, and 6% had four.

## DISCUSSION

Considerable attention has been given to antibiotic resistance found among pathogenic strains of bacteria causing disease in humans, other animals, and plants. The present study determined the relative frequency of potential reservoirs of these resistance genes residing in the fecal flora of humans at large. We examined ambulatory donors as well as hospitalized patients with and without a history of having taken antibiotics in the previous 2 weeks. This time period was selected because previous studies have shown that changes in fecal flora subjected to antibiotics were usually reversed by 10 to 14 days after stopping the drug (8, 14). In a second, more recent survey of urban dwellers, all individuals reported that they had not taken an antibiotic for 6 months. The findings in the two studies were similar.

More than 60% of the samples from those not taking an antibiotic showed resistance to at least one drug at a level of

TABLE 4. Resistance of Lac$^+$ flora in single- versus multiple-sample analyses

| Analysis and sample size | Frequency level (%) | Resistance (%)[a] | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | Ap | Sm | Tc | Kn | Cm | Gm | Na |
| Single sample | ≥10 | 42.2 | 36.0 | 34.4 | 22.3 | 4.3 | 5.6 | 0.9 |
| 636[b] | ≥50 | 24.0 | 20.3 | 22.3 | 7.2 | 2.2 | 0.5 | 0.5 |
| Multiple sample | ≥10 | 41.0 | 35.7 | 32.6 | 25.5 | 4.0 | 7.4 | 0.9 |
| 1,043 | ≥50 | 23.4 | 20.4 | 20.4 | 8.3 | 2.0 | 1.1 | 0.5 |

[a] For definitions of drug abbreviations, see footnote *c* to Table 2.
[b] Excludes four with no Lac$^+$ organisms.

≥10% of the total lactose-fermenting organisms (Table 2). Almost 40% of all samples from this group contained two or more different resistance determinants at this level (Fig. 4). Of these, much of the multiple resistance occurred in the same organism (Table 3). Common resistances were Ap$^r$, Sm$^r$, and Tc$^r$, followed closely by Kn$^r$. Cf$^r$ was examined in the hospital population and was also found to be high. Cm$^r$, Gm$^r$, or Na$^r$ appeared in <10% of the samples.

Using a variety of methods, other investigators in different countries have examined the frequency of resistance in fecal flora of smaller sample sizes of 25 to 100 donors. These studies were performed in the late 1960s and early 1970s primarily with hospital-associated personnel and patients with no known antibiotic ingestion (3, 15, 16, 18). While it is difficult to compare these studies due to differences in antibiotic selection and methodology, in general, they showed that about 20 to 40% of samples bore resistance to one or more drugs in 10% or more of the fecal flora (3, 15, 16, 18).

Only a small number of studies have examined healthy, ambulatory populations (11, 13, 17). The most extensive of these, performed in 1968 to 1970, examined 309 children and adults from urban and rural areas of England (11). Using both quantitative and qualitative methods, this group found that all samples contained some level (usually low) of organisms resistant to ampicillin, streptomycin, tetracycline, kanamycin, chloramphenicol, nalidixic acid, nitrofurantoin, or sulfafurazole. Only 17% of samples had resistance in isolates selected from the most common morphologic phenotype.

We found an even higher prevalence of resistance genes in the normal gut bacteria of people in the Boston area than was reported from these previous, more limited studies. Since we did not examine sulfonamide resistance, which in other studies occurred as frequently as tetracycline (18), our estimate of resistance frequency is probably lower than if this drug had also been tested. The low frequency of Gm$^r$, Na$^r$, and Cm$^r$ may reflect lower general usage of these drugs, but this does not explain the high frequency of Sm$^r$ in the face of minimal usage of this drug today (9) and in the population we studied (data not shown).

Previous reports have documented increased infection or gut colonization with resistant bacteria during hospitalization, with or without antibiotic treatment (3, 12, 15, 16, 19). One study observed an almost twofold increase in incidence of Cm$^r$ and Tc$^r$ strains in patients not taking antibiotics who were hospitalized for 2 weeks (19). The resistance frequency dropped after leaving the hospital. In the present study we found only a minimal difference in resistance to the individual drugs in fecal samples from the hospitalized individuals off antibiotics as compared with ambulatory groups (Table 2). Even when the presence of two or more resistance determinants in the sample was examined, hospitalized and ambulatory groups off antibiotics were not statistically different.

A history of recent antibiotic treatment among hospitalized patients correlated with a significant increase only in the frequency of Ap$^r$ and not resistance to the other drugs (Fig. 3). Although we also noted that Ap$^r$ was the only significant difference between noningesting hospitalized and ambulatory groups (Fig. 2), we believe this increase is linked to antibiotic ingestion since our comparison dealt with only the hospitalized group. Therefore, both hospitalization and antibiotic ingestion are involved in a statistically significant increase in Ap$^r$. We also observed a significant increase in the relative numbers of different antibiotic resistance determinants in each fecal sample of antibiotic ingestors (Fig. 4). Still, this antibiotic effect was less evident than the two to threefold changes reported in previous studies (3, 15, 16, 19). This may be related to an already higher base-line level of resistance in the population studied here. Antibiotic usage did not lead to a change in the lactose fermentation profile of the gut flora, demonstrating that resistance determinants in *E. coli* and other lactose-fermenting gut bacteria were sufficiently common that a change in relative distribution of the organisms did not occur.

Although most individuals showed a gain or loss of resistant bacteria when multiple samples were analyzed, there was no effect on the population as a whole (Table 4). The constant changing of the fecal *E. coli* population has been reported by others (2). Our findings suggest that these bacteria represent a large common pool of resistance determinants which are maintained at particular levels in people even from different family, residential, and occupational groups, while they may change within different fecal samples from the same individual.

To our knowledge, this is the first such extensive comparative evaluation on the frequency of antibiotic resistance genes in the gut flora of ambulatory and hospitalized people. Although the initial study was performed in the late 1970s, the recent sampling of members of one of the same populations (medical students) not ingesting antibiotics showed similar frequencies of both single and multiple resistance. Consequently, we believe that our earlier findings reflect the present-day frequency of antibiotic resistance in the fecal flora of an ambulatory population, in which the recent study showed that 7.5% of the samples had significant (≥10%) levels of trimethoprim resistance.

A major portion of the aerobic gram-negative gut flora (at least in the Boston area) now consists of resistant bacteria. About $10^6$ to $10^8$ *E. coli* are generally found per gram of human feces (10). Given about 100 g of feces excreted per day, an individual with a 10% frequency of a resistance determinant produces $10^7$ to $10^9$ resistant bacteria per day. Even at a 1% resistance level, relatively large amounts of resistance determinants are being excreted. This represents

a large reservoir of resistant bacteria and resistance genes. This situation may be the consequence of slow loss (i.e., more than 6 months) of resistance determinants acquired from previous antibiotic selection or by ingestion of resistant strains in foods (7) or by other unidentified factors which favor their persistence (6). Whatever the cause, these findings show the present-day carriage of high numbers of resistant bacteria and resistance genes in the human gut flora in the absence of concurrent or recent antibiotic consumption.

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