Human Therapeutic and Agricultural Uses of Antibacterial Drugs and Resistance of the Enteric Flora of Humans

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Fecal samples were collected from five groups of people differing in the manner of their exposure to antibacterial drugs. The groups included: (i) people working on farms who were continuously in contact with the predominantly resistant floras of farm animals receiving rations containing antibacterial drugs, (ii) people residing on the same farms with no direct exposure to the farm animals, (iii) people treated with antibacterial drugs, (iv) untreated people residing with treated individuals, and (v) untreated people with no exposure to farm animals or treated individuals. The samples were examined by quantitative plating for proportions of antibiotic-resistant, gram-negative enteric organisms. Individual isolates were also examined for their susceptibility to 11 different antibacterial drugs. The results indicate that enteric floras unexposed directly to the selective effects of antibacterial drugs may be affected by contact with predominantly resistant floras directly exposed to antibacterial drugs.

Antibacterial drugs are widely used for production purposes on livestock and poultry farms. This practice has been questioned because it might create conditions detrimental to animal and human health. It has been established that the enteric floras of farm animals are largely composed of drug-resistant organisms as a result of the continuous exposure to antibacterial agents (5, 7-10). Due to the transmissible nature of drug resistance in gram-negative enteric organisms, it has been postulated that the resident animal flora may comprise a reservoir of resistance for animal and human pathogens.

A central question to an objective assessment of the possible danger to public health posed by the agricultural use of antibacterial drugs is whether or not organisms and/or R factors originating on the farm can become established in the floras of human beings who have consumed food of animal origin. An investigation relating to this question was conducted to determine whether or not enteric floras continuously exposed to antibacterial drugs and composed largely of antibiotic-resistant organisms influenced epidemiologically associated enteric floras unexposed to antibacterial drugs. A previous paper reported the high proportions of antibiotic-resistant, gram-negative enteric organisms in fecal samples from Illinois farm animals given feeds containing antibacterial drugs (7). This paper reports the proportions of resist-

ant organisms in fecal samples from the following: human beings working and residing on some of the same Illinois farms, people recently treated with antibacterial drugs, co-inhabitants of treated individuals, and human beings having no exposure to antibacterial drugs and no known contact with treated individuals or Illinois farm animals.

MATERIALS AND METHODS

Media. MacConkey agar (Difco) was used for the determination of proportions of antibiotic-resistant, gram-negative enteric organisms in fecal samples. EMB agar (Difco) was used for secondary isolation of strains selected from MacConkey agar plates spread with fecal suspensions. Mueller-Hinton agar (BBL) was used for antibacterial drug susceptibility testing. Dilutions of fecal specimens were made in sterile saline (0.85% NaCl). Trypticase soy broth (BBL) was used for all broth cultures. Nutrient agar (Difco) slants were used for isolate storage.

Antibiotics. The following concentrations of antibacterial drugs in MacConkey agar were used: nalidixic acid, 40 µg/ml; oxytetracycline, 20 µg/ml; dihydrostreptomycin, 25 µg/ml; and ampicillin, 50 µg/ml. Concentrations of oxytetracycline, dihydrostreptomycin, and ampicillin were selected to provide the best possible correlations between Kirby-Bauer disk susceptibility tests and growth on agar.

Fecal samples. Ten- to 20-g fecal samples were collected into 40-ml quantities of sterile, pH 7.2, buffered-glycerol-saline solution (7) contained in small glass vials. The samples were sent to the laboratory in double-lined mailing containers.

Quantitation of drug-resistant, gram-negative enteric organisms. The procedure used to determine the relative proportions of oxytetracycline-,

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dihydrostreptomycin-, or ampicillin-resistant organisms has been described in detail (7). This procedure consisted of plating saline dilutions of a fecal suspension on MacConkey agar and MacConkey agar containing antibiotic. After overnight incubation at 37 C, colony counts were used to determine the total concentration of gram-negative enteric organisms present in the initial sample suspension and the concentrations of organisms resistant to each of the three antibiotics. If organisms resistant to one of the antibiotics could not be detected after plating fewer than 10^6 organisms, results for that antibiotic and sample were excluded.

Isolation and identification of gram-negative enteric isolates. From one to 10 colonies were obtained from antibiotic-free MacConkey agar plates spread with a fecal suspension of each sample. The selection of individual colonies was based upon easily recognizable differences in colonial morphology and differences in their utilization of lactose. Each colony was streaked on EMB agar. After overnight incubation, a single clone from the EMB agar plate was used to inoculate various biochemical test media and a nutrient agar slant. Inocula of broth cultures for antibacterial drug susceptibility testing were obtained from the nutrient agar slant. Isolates were identified according to the methods of Edwards and Ewing (3).

Antibacterial drug susceptibility testing. Antibacterial drug susceptibility testing was performed using high potency sensitivity disks (BBL) as described by Bauer et al. (1). Isolates with intermediate resistance were considered susceptible for the purposes of this investigation.

Exposure of human beings to antibacterial drugs. Fecal samples were arranged into one of five groups according to the direct or indirect exposure of each person to antibacterial drugs. Group 1 consisted of 22 individuals working on 16 farms utilizing antibacterial drug feed additives. These individuals had direct contact with farm animals and their flora. Samples from farm residents claiming to have occasional direct contact with farm animals were included among group 1 samples. Fourteen of the farms raised swine and two raised beef cattle and calves. Group 2 consisted of 20 individuals residing on 13 farms who had no direct contact with farm animals; 10 of the farms raised swine, two beef cattle and calves, and one poultry. Individuals in this group were infants, elderly persons, and housewives. None of the individuals in groups 1 and 2 had been medicated with antibacterial drugs in the preceding 6 months. Groups 1 and 2 represented 20 farms. Group 3 consisted of people who had received antibacterial medication for the treatment of salmonellosis within 3 weeks of the time the fecal sample was collected. Samples in group 4 were obtained from people who resided in the same homes as those in group 3, but had not received antibacterial medication within 6 months. Group 5 samples were obtained from individuals who had not received any type of medication during the 6 months prior to sample collection and lacked contact with farm animals as well as contact with medicated people.

Statistical analyses of proportions of antibiotic-

resistant organisms. To perform statistical analyses, the frequencies of organisms resistant to each antibiotic were converted to natural logarithms, and 20 U was added to each logarithm to convert the negative logarithms to positive values. The transformed values for each human group and each antibiotic were used in an analysis of variance (Student's *t* test with significant F).

RESULTS

Antibacterial drugs used as animal feed additives and for treatment of human salmonellosis. Of the 20 farms studied, 14 used chlorotetracycline as feed additive, in addition to penicillin G and a sulfonamide in most cases. One of the 14 farms used tylosin in addition of chlortetracycline, penicillin, and streptomycin. One of the swine farms used a nitrofur derivative and tylosin in addition to penicillin and streptomycin. Each drug was utilized at concentrations ranging from 50 to 100 g/ton.

The 18 medicated people of group 3 all had salmonellosis, and approximately one-third had been hospitalized. None were hospitalized at the time of sample collection. In a few cases, physicians would not reveal the identity of the specific antibacterial drugs prescribed. Ampicillin was administered in 10 of 14 cases. Two of the individuals treated with ampicillin also received chloramphenicol; another received a triple sulfonamide combination in addition to ampicillin. Tetracyclines were utilized in the remaining four cases, with one individual receiving tetracycline in combination with a sulfonamide effective in the intestinal tract and neomycin. Ten of the 18 treated individuals were not receiving antibacterial drugs at the time of sample collection.

Proportions of the gram-negative enteric flora resistant to antibiotics. Fecal samples from the five groups of individuals were examined for the proportions of the gram-negative enteric flora resistant to each of three antibiotics: oxytetracycline, dihydrostreptomycin, and ampicillin. The results of quantitating the proportions of oxytetracycline-resistant organisms are shown in Table 1.

The results are presented as the cumulative percentages of samples which contained equal to or greater than specific numbers of antibiotic-resistant organisms per 100,000 colony-forming units (CFU). In Table 1, the cumulative distributions of samples from groups 1, 2, and 3 were similar. Greater percentages of group 1, 2, and 3 samples contained higher proportions of oxytetracycline-resistant organisms: between 50 and 62% of the samples from groups 1, 2, and 3 contained 10,000 or more oxytetracycline-resistant organisms per 100,000

TABLE 1. Cumulative distributions of fecal samples from human groups according to proportions of oxytetracycline-resistant organisms

Determinants	Samples ^a (cumulative %)				
	Group 1	Group 2	Group 3	Group 4	Group 5
Resistant organisms per 100,000 CFU					
100,000	0	5	6	3	0
≥10,000	62	50	56	37	13
≥1,000	76	85	78	58	31
≥100	86	90	78	68	41
≥10	91	100	89	76	56
≥1	95	100	95	79	63
Samples with less than 1 resistant organism per 100,000 CFU	5	0	5	21	37
Total no. of samples	21	20	18	38	32

^a Groups as described in the text.

CFU. Group 4 samples less frequently contained higher proportions of oxytetracycline-resistant organisms: 37% contained 10,000 or more oxytetracycline-resistant organisms per 100,000 CFU, and 21% contained less than one oxytetracycline-resistant organism per 100,000 CFU. Group 5 samples were least likely to contain resistant organisms: 13% contained 10,000 or more oxytetracycline-resistant organisms and 37% contained less than one oxytetracycline-resistant organism per 100,000 CFU.

Statistical analysis of these results revealed the following: the frequency of oxytetracycline-resistant organisms in group 5 samples was significantly lower ($P < 0.05$) than the frequencies of each of the first four groups. Concomitantly, comparisons among groups 1 through 4 for prevalence of oxytetracycline resistance yielded no statistically significant differences.

The intergroup relationships for proportions of dihydrostreptomycin resistance observed in Table 2 were similar to those observed for oxytetracycline resistance in Table 1. Between 53 and 59% of the samples from groups 1, 2, and 3 contained greater than 10,000 dihydrostreptomycin-resistant organisms per 100,000 CFU. Samples from group 5 infrequently contained high proportions of dihydrostreptomycin-resistant organisms, whereas samples from group 4 generally demonstrated proportions of dihydrostreptomycin-resistant organisms intermediate between those of groups 3 and 5.

Statistical analysis of the frequencies of dihydrostreptomycin-resistant organisms of each

group again revealed significantly lower ($P < 0.05$) frequencies in samples from group 5 than in samples from groups 1 through 4. There were no statistically significant differences in frequencies of dihydrostreptomycin-resistant organisms among groups 1 through 4.

The distributions of samples according to proportions of ampicillin-resistant organisms are presented in Table 3. The intergroup relationships found in determinations of proportions of

TABLE 2. Cumulative distributions of fecal samples from human groups according to proportions of dihydrostreptomycin-resistant organisms

Determinants	Samples ^a (cumulative %)				
	Group 1	Group 2	Group 3	Group 4	Group 5
Resistant organisms per 100,000 CFU					
100,000	5	16	0	3	0
≥10,000	59	58	53	38	10
≥1,000	68	63	71	62	28
≥100	82	84	71	73	34
≥10	95	90	76	78	45
≥1	95	100	88	84	65
Samples with less than 1 resistant organism per 100,000 CFU	5	0	12	16	35
Total no. of samples	22	19	17	37	29

^a Groups as described in the text.

TABLE 3. Cumulative distributions of fecal samples from human groups according to proportions of ampicillin-resistant organisms

Determinants	Samples ^a (cumulative %)				
	Group 1	Group 2	Group 3	Group 4	Group 5
Resistant organisms per 100,000 CFU					
100,000	0	6	7	0	0
≥10,000	67	47	33	23	0
≥1,000	72	65	47	40	14
≥100	86	82	47	53	32
≥10	91	94	53	60	36
≥1	91	100	80	70	61
Samples with less than 1 resistant organism per 100,000 CFU	9	0	20	30	39
Total no. of samples	21	17	15	30	28

^a Groups as described in the text.

ampicillin-resistant organisms were similar to those found in determinations of proportions resistant to oxytetracycline or dihydrostreptomycin. Although 20%, or three, of the group 3 samples contained less than one ampicillin-resistant organism per 100,000 CFU, these samples were obtained from three of the four persons who were not treated with ampicillin, but received a tetracycline.

Statistically significant differences ($P < 0.05$) in frequencies of ampicillin-resistant organisms occurred between group 5 and groups 1, 2, and 4. Although significant statistical difference was established between group 4 and group 5, no significant difference in the frequencies of ampicillin-resistant organisms occurred between group 3 and group 5. The distributions of frequencies in groups 3 and 4 were almost identical; however, there were too few samples in group 3, which limited the degrees of freedom, to demonstrate any statistically significant difference between group 3 and group 5. No significant differences occurred when frequencies of ampicillin-resistant organisms from groups 2, 3, and 4 were compared. Frequencies from group 1 were significantly higher than those from the other groups with the exception of group 2.

Antibacterial drug resistances. Table 4 presents *Escherichia coli* susceptibilities to 11 different antibacterial drugs. The occurrence of resistance to nalidixic acid, colistin, nitrofurantoin, chloramphenicol, and gentamicin was uniformly low. Oxytetracycline, dihydrostreptomycin, and ampicillin resistance occurred most

frequently among *E. coli* from samples of groups 1, 2, and 3. The frequency of resistance to these three drugs was lowest for samples from group 4 (22% oxytetracycline, 19% dihydrostreptomycin, and 10% ampicillin) and occurred slightly more frequently among *E. coli* from group 5 than group 4. Thirty-three percent of the group 5 isolates were resistant to oxytetracycline, 28% to dihydrostreptomycin, and 13% to ampicillin.

Resistance to neomycin was highest in *E. coli* from groups 1, 2, and 3, ranging between 12 and 19% of the isolates. Resistance to neomycin was absent among *E. coli* of group 5 and was minimal among *E. coli* of group 4. Cephalothin resistance occurred infrequently among *E. coli* from samples of groups 1, 2, 4, and 5, whereas 21% of the *E. coli* from group 3 were resistant to cephalothin. Resistance to sulfamethazine occurred in 51% of the *E. coli* of group 1 and 42% of the *E. coli* from group 2. Resistance to sulfamethazine among *E. coli* from the remaining three groups was lower, ranging between 10 and 16% of the isolates.

Table 5 presents the susceptibilities of all gram-negative enteric isolates to various antibacterial drugs. Resistance to nalidixic acid, colistin, chloramphenicol, and gentamicin was uniformly low for isolates from all five groups. The frequencies of resistance to oxytetracycline and dihydrostreptomycin were similar for isolates from groups 1, 2, and 3, ranging between 33 and 49%. The frequency of oxytetracycline- and dihydrostreptomycin-resistant isolates

TABLE 4. Antimicrobial drug resistances of *E. coli* from human groups with varying exposure to the selective effects of antibacterial drugs

Antibacterial drugs	Isolates resistant to each antibacterial drug (%)				
	Group 1	Group 2	Group 3	Group 4	Group 5
Nalidixic acid	0	4	7	0	0
Oxytetracycline	49	62	50	22	33
Dihydrostreptomycin	60	53	43	19	28
Ampicillin	33	28	37	10	13
Colistin	0	5	0	0	4
Neomycin	12	19	17	1	0
Nitrofurantoin	4	2	0	1	4
Chloramphenicol	0	2	0	0	0
Gentamicin	0	0	0	0	0
Cephalothin	4	0	21	2	5
Sulfamethazine	51	42	14	16	10
Total no. of isolates	57	53	30	117	40

TABLE 5. Antibacterial drug resistance of all gram-negative enteric isolates from human groups with varying exposure to the selective effects of antibacterial drugs

Antibacterial drugs	Isolates resistant to each antibacterial drug (%)				
	Group 1	Group 2	Group 3	Group 4	Group 5
Nalidixic acid	1	3	3	3	0
Oxytetracycline	35	49	35	18	24
Dihydrostreptomycin	43	41	33	20	19
Ampicillin	42	36	53	42	24
Colistin	2	3	0	5	2
Neomycin	8	13	6	5	0
Nitrofurantoin	4	3	12	6	3
Chloramphenicol	0	3	0	3	0
Gentamicin	0	0	0	0	0
Cephalothin	19	8	68	43	11
Sulfamethazine	43	41	7	9	10
Total no. of isolates	86	76	87	225	62

from groups 4 and 5 were lower than for groups 1, 2, and 3, ranging between 18 and 24%.

Ampicillin resistance was most frequent (53%) among isolates from group 3 and was least frequent (23%) among isolates from group 5. Similar frequencies (36 to 42%) of ampicillin-resistant organisms were found in groups 1, 2, and 4. Cephalothin resistance was most frequent among isolates of group 3 (68%) and less frequent among isolates from group 4 (43%). Cephalothin resistance was least frequent among isolates from groups 1, 2, and 5. Neomycin resistance was absent among isolates of group 5 and ranged from 5 to 13% in the remaining groups. Sulfamethazine resistance was the most frequent for groups 1 and 2 (43 and 41%) as compared to the resistance for the remaining three groups (between 7 and 10%).

DISCUSSION

Samples from persons recently treated with antibacterial drugs (group 3) and samples from persons working and/or living on farms (groups 1 and 2) contained the highest proportions of gram-negative enteric organisms resistant to oxytetracycline, dihydrostreptomycin, and ampicillin. Resistance to these antibiotics was also most frequent among *E. coli* and all gram-negative enteric isolates from groups 1, 2, and 3. Had all samples of group 3 been obtained from persons receiving medication at the time of sample collection, higher frequencies and a greater prevalence of resistance among isolates of group 3 might have been found.

The presence of ampicillin-resistant organisms in farm animals (7) was reflected in the appearance of ampicillin-resistant organisms in the farm worker and resident. Although ampicillin was not used on the farm, the use of other antibacterial drugs in animal feed appeared to result in the emergence of ampicillin-resistant organisms (7). Kerrebijn et al. (4) noted that the human use of azidocillin, an antibiotic which is similar to penicillin G in its low activity against gram-negative enteric organisms, was associated with an increased prevalence of ampicillin resistance; it was suggested that exposure of organisms to the 6-aminopenicillanic acid nucleus, which is present in all penicillin antibiotics including azidocillin, was responsible for the emergence of ampicillin resistance. Penicillin G was commonly used on the farm.

Group 5 fecal samples contained the lowest proportions of antibiotic-resistant organisms. The proportions of resistant organisms in samples from group 4 were intermediate between the higher proportions observed for group 3 and

the relatively lower proportions seen for samples from group 5. The prevalence of resistance to specific antibacterial agents among *E. coli* and all gram-negative enteric isolates from groups 4 and 5 was lower than that of the first three groups. Although ampicillin and cephalothin resistance was less frequent among all gram-negative enteric organisms from group 5 compared to group 4, the absence of differences in the prevalence of resistance in all other instances between the two groups may be accounted for by the limited number of isolates examined.

Cephalothin resistance appeared most frequently in isolates from group 3 and less frequently in isolates from group 4. Some of the β -lactamases produced by gram-negative enterics are active against both ampicillin and cephalosporins and consequently confer cross-resistance to these agents (6). Ampicillin was the most commonly used antibacterial drug in group 3 persons. It is speculated that the activity of ampicillin against gram-negative enterics and the occurrence of cross-resistance between ampicillin and cephalosporins might account for the increased prevalence of cephalothin resistance in groups 3 and 4. The absence of the same degree of activity of penicillin G on gram-negative enterics could explain the lower prevalence of resistance to cephalothin in groups 1 and 2 and the farm animals (7).

The use of sulfonamides on the farm was reflected in a higher prevalence of sulfamethazine resistance in *E. coli* and all gram-negative enteric organisms from groups 1 and 2. The occasional therapeutic use of neomycin in farm animals and its use with group 3 humans was reflected in a slightly increased prevalence of neomycin resistance among isolates from groups 1, 2, and 3.

In this study, no attempts were made to control accidental inhalation or ingestion of drug-supplemented animal feed or to determine whether such inhalation could influence the prevalence of resistant organisms in the flora of the farmer. The highest concentration of any single antibacterial drug in animal feeds in this study was 100 g/ton. The most commonly used combination contained 100 g of chlortetracycline per ton, 100 g of sulfamethazine per ton, and 50 g of penicillin G per ton. Substantial amounts of feed thus would have to be continuously inhaled or ingested to maintain fecal drug concentrations necessary to exert selective effects on the fecal flora of the farmer.

The enteric flora of human beings in contact with farm animals or medicated people contain greater frequencies of resistant organisms than

do florae of people unexposed to farm animals or medicated people. Assuming that the direct effects of drugs in feed upon the florae of farm workers and/or residents was minimal, the observations made in this study support the contention that the exposure of one enteric flora to antibacterial drugs, and the consequent emergence of resistant organisms, may alter the composition of epidemiologically adjacent enteric florae unexposed to antibacterial drugs.

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