

Drug-Resistant *Escherichia coli* and *Klebsiella-Enterobacter* in Healthy Adults in Thailand and the Effect of Antibiotic Administration

CHAWEWAN KOONKHAMLERT AND WILLIAM D. SAWYER

Department of Microbiology, Faculty of Science, Mahidol University, and The Rockefeller Foundation,
Bangkok, Thailand

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Antibiotic-resistant strains of *Escherichia coli* and, to a lesser extent, *Klebsiella-Enterobacter* were common in serial samples of the normal intestinal flora of healthy young adults in Thailand, and the resistance often was to multiple drugs and was transferable. Antibiotic administration promoted dominance of the flora by drug-resistant organisms regardless of the drug regimen and the predrug susceptibility pattern of the flora. The normal pattern was regained slowly after the cessation of drug administration.

In Thailand, pathogenic bacteria that are isolated from patients frequently contain an R factor(s) that confers resistance to multiple antibiotics (8), and *Escherichia coli* strains with infectious drug resistance are commonly present in the fecal flora of healthy children (7) and adults (9).

To study the dynamics of healthy carriage of drug-resistant enterobacteria, we obtained serial samples from the intestinal flora (rectal swab), the skin (hand washings in sterile saline), and the respiratory tract (throat swab) of 21 healthy young adults. Except for one participant who took tetracycline therapeutically for a brief period after a sample and ending 10 days before the next sample, the people received no antimicrobial agents during this portion of the study. Samples were cultivated on eosin-methylene blue agar (Difco). After growth, up to 20 colonies typical of *E. coli* and up to 15 colonies resembling *Klebsiella-Enterobacter* were isolated and identified by standard fermentation reactions (14).

Each isolate was tested for susceptibility (9) first to 100 μg of nalidixic acid/ml (Sterling-Winthrop) in tryptic soy agar (TSA; Difco) and, if susceptible, then in TSA to 12.5 μg of each of the following/ml: ampicillin (Bristol Laboratories), chloramphenicol (Carlo Erba), kanamycin (Bristol Laboratories), neomycin (Roussel Uclaf), streptomycin (Hoechst), and tetracycline (Lederle Laboratories). Nalidixic acid-

resistant isolates, <0.05% of the total, were excluded. Because nearly all *Klebsiella-Enterobacter* strains resisted the test concentration of ampicillin, these results were also excluded. All other resistant organisms were tested once for capacity to transfer resistance (9) to an *E. coli* K-12 strain that (i) was free from known episomes, (ii) resisted 100 μg of nalidixic acid/ml, and (iii) was susceptible to the other antibiotics tested. After growth of a test donor and the recipient as a mixed culture for 2 h, the recipient *E. coli* cells were first selected on TSA with 100 μg of nalidixic acid/ml and then were tested as above for acquired resistance to the antibiotics that the donor resisted. Controls were a standard donor with the recipient, the recipient alone, and the test donor alone. The results with duplicate rectal swabs from five people demonstrated that both the proportion of drug-resistant *E. coli* cells and the pattern of drug susceptibility were similar in the two samples. Therefore, a single sample was processed at each collection.

In 8 to 11 weekly or biweekly samples during 15 to 17 weeks (Table 1), *E. coli* strains that resisted one or more of the drugs were (i) recovered from the fecal flora of each person at some time, (ii) present in a majority of the fecal samples from the individuals, and (iii) recovered from 86% of the total fecal samples (182 of 211). Resistant *E. coli* strains comprised \geq 50% of the fecal *E. coli* isolates in at least one

sample from most of the people (Table 1) and in 23% of all samples (48 of 211). The samples frequently contained *E. coli* strains that resisted more than one of the drugs (126 of 211 samples, 60%) and that could transfer drug resistance (122 of 211 samples, 58%). Of the 3,142 isolates of *E. coli* that were tested in this portion of the study (Table 2), 31% resisted at least one of the drugs, 15% resisted more than one of the drugs, and 12% transmitted resistance. Resistance was most common to tetracycline (27% of total isolates) followed by streptomycin (16%), ampicillin (6%), chloramphenicol (5%), and kanamycin (1%) and neomycin (1%).

Klebsiella-Enterobacter strains were recovered from the fecal flora less often than *E. coli* strains (Table 1). A majority of the persons had drug-resistant *Klebsiella-Enterobacter* strains in the feces at some time; most of these individuals carried the drug-resistant organisms only occasionally. Of the 189 samples, *Klebsiella-Enterobacter* strains were recovered that resisted one or more drug from 31 (16%), that resisted more than one drug from 18 (10%), and that transferred resistance from 23 (12%). A small proportion of the total isolates of *Klebsiella-Enterobacter* resisted drugs and transferred resistance (Table 2). Resistance was mainly to tetracycline and, to a lesser extent, to streptomycin.

Occasionally, a few *E. coli* cells were recovered from the respiratory tract (1 sample from three different people) and a few *Klebsiella-Enterobacter* cells were recovered from the skin (five samples from four people) and respiratory tract (13 samples from four people); *E. coli* was not recovered from the respiratory tract. Except for one isolate of *E. coli* that re-

TABLE 2. Drug resistance in strains of *E. coli* and *Klebsiella-Enterobacter* isolated from serial samples of the fecal flora of 21 healthy adults during 15 to 17 weeks

Criterion	No. of isolates	
	<i>E. coli</i>	<i>Klebsiella-Enterobacter</i>
Tested	3,142	965
Resistant to ≥ 1 drug ...	986 (31%) ^a	68 (7%)
Resistant to > 1 drug ...	466 (15%)	28 (3%)
Transferred resistance ..	367 (12%)	38 (4%)

^a Percentage of total isolates.

sisted tetracycline, the extraintestinal isolates of both organisms were susceptible to the drugs used here.

The effect of antibiotic administration on the healthy carriage of *E. coli* and *Klebsiella-Enterobacter* was assessed in 20 of the people who volunteered to take antibiotics; they were randomly divided into four groups of five, and each group was assigned to one of the following regimens of orally administered antibiotics: (i) ampicillin, 250 mg thrice daily; (ii) neomycin, 250 mg thrice daily; (iii) tetracycline, 250 mg four times daily; or (iv) both neomycin and tetracycline in the above daily doses. Sampling was conducted as before at 3 and 5 days of administration and at 3 days and 1, 2, 3, and 4 weeks after administration of the antibiotics. By 3 days after the start of the drug, the volunteers uniformly had drug-resistant *E. coli* cells in their fecal flora, and all of the *E. coli* isolates were drug-resistant regardless of the drug regimen and the drug-susceptibility patterns of the fecal *E. coli* isolates before the drug. The *E. coli* strains resisted the drug(s) consumed and, commonly, three to five of the other drugs. Most isolates transferred resistance in the single test of transfer capacity. This pattern persisted for a variable time after drug administration, but gradually the members of each of the groups regained a pattern typical of the subjects in the predrug period. The return to the typical "normal" pattern was similar in the four groups and required 3 to 4 weeks.

The effect of antibiotic consumption on the *Klebsiella-Enterobacter* strains of the intestinal flora resembled that on *E. coli*. During drug administration, *Klebsiella-Enterobacter* strains with infectious multiple drug resistance dominated the *Klebsiella-Enterobacter* flora regardless of the drug(s) administered and the susceptibility of the predrug flora. The typical "normal" pattern was gradually restored after cessation of the drug regimen.

TABLE 1. Drug-resistant strains of *E. coli* and *Klebsiella-Enterobacter* in 7 to 12 samples of the fecal flora of each of 21 healthy adults during 15 to 17 weeks

Criterion	No. of persons with drug-resistant	
	<i>E. coli</i>	<i>Klebsiella-Enterobacter</i>
Detected	21 (100%) ^a	16 (76%)
Detected in		
100% of samples	8 (38%)	0 (0%)
75 to <100% of samples ...	9 (43%)	0 (0%)
50 to <75% of samples ...	4 (19%)	1 (5%)
25 to <50% of samples ...	0 (0%)	3 (14%)
>0 to <50% of samples ...	0 (0%)	12 (57%)
Detected as ≥ 50% of isolates in		
≥ 1 sample	18 (86%)	11 (52%)
≥ 50% of samples	3 (14%)	0 (0%)

^a Percentage of 21 people.

Drug administration did not promote colonization of the skin and respiratory tract by either *E. coli* or *Klebsiella-Enterobacter* in these individuals.

Thus, these studies emphasize the ready acquisition of drug-resistant enteric bacteria by residents of Thailand both normally and during the selective pressure of antibiotic administration. Differences in the populations studied and in the techniques used complicate comparison of the prevalence of drug-resistant strains of *E. coli* and *Klebsiella-Enterobacter* in healthy people in various parts of the world; some examples, however, may help to put the situation in Thailand in perspective. In the early 1960's in Japan, only about 1.4% of people in a large sample had drug-resistant *E. coli* strains in their fecal flora (15). By 1966, however, 45% of *E. coli* isolates and 43% of *Klebsiella* strains were reported to have infectious drug resistance (15). In Western Europe in the past decade, the prevalence of drug-resistant *E. coli* strains in the fecal flora of normal adults has been reported over a range of 21 to 62% with many of the results falling in the range of 21 to 29% (2, 6, 10, 11, 16). In further contrast to the results in Thailand, the proportion of drug-resistant organisms in the fecal *E. coli* flora was low, exceeding 10% in only 20% of people (6). A recent study from the United States described drug resistance in 54% of *E. coli* isolates from healthy students (1). A prevalence of 78% in an urban population in South Africa (13) approximates the present results. In the above and the present studies, the pattern of drug resistance was similar: frequently multiple, often transferable, and involving tetracycline and streptomycin most commonly. The prevalence of drug-resistant *Klebsiella-Enterobacter* strains seems low generally (e.g., 4). Resistance to ampicillin is common, as noted herein, and such resistance is often not transferable. The *Klebsiella-Enterobacter* isolates most commonly resist streptomycin and tetracycline among other antibiotics.

Among factors that may contribute to the rapid circulation of drug-resistant enterobacteria in the population in Thailand and other areas of high prevalence are (i) heavy environmental contamination by fecal organisms, e.g., in foods and water (17), (ii) crowded living conditions, and (iii) the constant and strong selective pressure exerted through widespread usage of antibiotics both by prescription and by ready availability "over the counter." The impact of great antibiotic usage is emphasized by the results of antibiotic administration and also by the striking contrast in the prevalence of

drug-resistant fecal *E. coli* isolates between drug-using (above) and drug-free societies, e.g., 3% in Borneo (3), 5% in the Solomon Islands (5), and 19% in South Africa (12)—areas in which fecal contamination of food and waters is probably high.

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