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Emergence of Resistant Fecal *Escherichia coli* in Travelers Not Taking Prophylactic Antimicrobial Agents

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Fecal specimens from individuals traveling to Mexico were examined before, during, and after travel for the presence of *Escherichia coli* resistant to ampicillin, chloramphenicol, gentamicin, kanamycin, streptomycin, sulfonamides, trimethoprim (TMP), and TMP-sulfamethoxazole (TMP-SMX). None of these individuals took prophylactic antibiotics, although 4 of 13 took short courses of an antimicrobial agent for therapy of traveler's diarrhea. With an average of 9.3 *E. coli* per sample, resistance to all agents tested except gentamicin was shown to increase during the time in Mexico ($P < 0.001$ to $P < 0.05$). For example, no TMP-resistant (Tmp^r) *E. coli* isolates were found by this method before travel, whereas 57% of the individuals had Tmp^r and Tmp^r-Smx^r *E. coli* by the final week in Mexico. This increase in resistance occurred regardless of whether an individual took a short course of antimicrobial therapy. This study shows that travel itself, even without the use of prophylactic or therapeutic antimicrobial agents, is associated with the acquisition of resistant *E. coli*. Travel to developing nations may rival other sources of resistant organisms.

A number of studies have established that travel to developing countries is associated with an increased risk of diarrheal illness, presumably caused by ingestion of enteric pathogens (7, 19). We have also shown that travelers to Mexico who consumed prophylactic trimethoprim (TMP) or TMP-sulfamethoxazole (TMP-SMX), but not placebo, for three weeks while in Mexico had their TMP-susceptible fecal *Escherichia coli* replaced by TMP-resistant *E. coli*; since this was not observed in a similar study in the United States, the emergence of resistance in travelers to Mexico was felt to be related to increased exposure to resistant organisms as well as to the selective pressure exerted by the antibiotics (12). We have now studied fecal *E. coli* from travelers to Mexico who were not taking prophylactic antibiotics; the goal was to determine if travel to a developing region, even without antibiotic therapy, is associated with the emergence of antibiotic-resistant fecal *E. coli*.

MATERIALS AND METHODS

Study participants included 13 residents of the United States traveling to Guadalajara, Mexico, in 1987. Fecal specimens were obtained from the participants before departure (week 0); during weeks 1, 2, 3, and 6 while in Mexico; and after returning to Houston, Tex. (week 7). Fresh stool specimens were processed in two ways. First, a sample of approximately 1 mg was streaked onto MacConkey agar to obtain isolated colonies and incubated overnight at 37°C. As many as 10 isolated *E. coli*-like colonies were picked (fewer were picked only if 10 separate colonies could not be found) and inoculated into peptone stabs for transport back to Houston. This method is referred to as the 10-colony method. A second sample was streaked onto PW agar (per liter: 40 g of tryptic soy agar, 10 g of lactose, 1.5 g of bile salts, 0.001 g of crystal violet, 0.03 g of neutral red, and 50 IU of thymidine phosphorylase) containing 50 μg of TMP per ml. This agar, like MacConkey agar, is selective for gram-negative bacteria but does not have the problem of breakthrough growth that is seen when TMP is tested with MacConkey agar (15, 21). Representative *E. coli*-like colonies growing on PW with TMP were also inoculated into peptone stabs and transported to Houston. This method is referred to as the plate detection method. Colonies were subsequently identified in our laboratories by routine biochemical methods and tested for antibiotic susceptibility to ampicillin (AP), chloramphenicol (CM), gentamicin (GM), kanamycin (KM), streptomycin (SM), sulfonamides (SU), tetracycline (TC), TMP, and TMP-SMX by the disk diffusion method (13). Only those isolates identified as *E. coli* are reported here. Both the percentages of persons with *E. coli* isolates resistant to the individual agents and the percentages of resistant isolates were calculated.

In order to determine an overall measure of resistance, a resistance score was calculated by the following equation: resistance score = (total number of resistances per total number of possible resistances) × 100. The total number of resistances is the summation of the number of colonies resistant to each antibiotic; the total number of possible resistances is the total number of colonies tested (approximately 10 per specimen) times the number of antibiotics tested. The significance of the trend for increasing resistance to individual agents and the significance of the increase in the resistance score were determined by the Armitage test for trend (1).

RESULTS

Specimens obtained. A total of 66 stool specimens were studied. Stool samples were available from all 13 participants in weeks 0, 1, and 2; from 11 individuals in week 3; from 9 individuals in week 6; and from 7 individuals in week 7 (Table 1). Four of the participants took antibiotics at some time during the study. One individual took TMP-SMX for 3 days in week 2 and norfloxacin for 3 days in week 3; one took 1 dose of TMP-SMX in week 1 and norfloxacin for 3 days in week 6; one took TMP-SMX for 3 days in both weeks 1 and 2; and one took ofloxacin for 3 days in week 6 (Table 1). The

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TABLE 1. Location and timing of stool sample collections

Wk	Location	Total no. of persons supplying fecal sample	Antibiotic (no. of participants)[a]
0	Houston	13	
1	Guadalajara	13	TMP-SMX (2)
2	Guadalajara	13	TMP-SMX (2)
3	Guadalajara	11	Norfloxacin (1)
6	Guadalajara	9	Ofloxacin (1) Norfloxacin (1)
7	Houston	7	

[a] Four participants took a total of seven courses of antibiotics; see text for details.

TABLE 3. Resistance scores

Wk	Resistance score (%) for participants[a]		
	No antibiotics	Antibiotics	All
0	13	11	12
1	18	7	15
2	26	34	28
3	32	30	32
6	35	38	36
7	33	59	40

[a] Calculated as total number of resistances/total number of possible resistances) × 100. The trend for increasing resistance was highly significant ($P \ll 0.0001$). See also Table 1. Of the 13 individuals in the study, 4 took antibiotics at some time during the study; these 4 were analyzed separately from the 9 who did not take antibiotics during the study.

other participants had no diarrhea and did not take antibiotics.

Ten-colony method. A total of 617 isolates of *E. coli* were selected from the 66 stool specimens submitted (average, 9.3 colonies per sample). The numbers of isolated colonies that were obtained from the specimens were as follows (per specimen): 10 from 42 specimens; 9 from 13 specimens; 8 from 5 specimens; 7 from 4 specimens; and 6, 5, and 1 from 1 specimen each. By using the results of susceptibility testing of the individual colonies, the percentages of all persons with at least one colony resistant to the individual antimicrobial agents tested were calculated (Table 2). The percentages of persons with resistance to AP, SM, SU, and TC had markedly increased by week 2 and had increased for all antibiotics except GM by week 6. The trend for an increasing percentage of persons with resistance to an antimicrobial agent was significant for AP and CM (one-tailed $P < 0.05$), for KM, SU, and TC (one-tailed $P < 0.01$), and for SM, TMP, and TMP-SMX (one-tailed $P < 0.001$) and was not significant for GM. Almost identical results were obtained when only individuals receiving no antibiotics were analyzed (Table 2). For example, in week 6, the percentages of individuals with resistance who took no antibiotics were 43% for AP, 29% for KM, 0% for GM, 86% for SM, 100% for SU, 86% for TC, and 57% for both TMP and TMP-SMX; these results are within 1 to 4 percentage points of the results listed in Table 2 for all individuals.

Resistance scores were determined for all persons, for persons receiving antibiotic therapy, and for persons receiving no antibiotic therapy (Table 3). In all groups, the resistance score had increased by twofold or more by week 2. The trend for increasing resistance was highly significant, with one-tailed $P \ll 0.0001$ by the Armitage test for trend. Only AP, CM, SU, TC, and TMP were used in this analysis, since these are oral agents that are commonly used; when SM, KM, and GM were included, the trends and conclusions were the same.

Another measure of resistance that we evaluated was the percentages of persons with multiple resistances among their predominant *E. coli*. The numbers for multiresistance reflect either multiresistant isolates or different isolates from the same individual resistant to different agents. Before travel, 15% of the participants had *E. coli* isolates that were either individually or collectively resistant to three or more antimicrobial agents and 76% had an *E. coli* isolate resistant to at least one antibiotic (Fig. 1). By week 2, resistance to three or more agents was found among the *E. coli* isolates of 70% of those persons tested and 93% had resistance to at least one agent; these percentages remained relatively stable throughout the remainder of the study. As described in the preceding section, only agents administered orally were evaluated, but the results, which included those for aminoglycosides, showed the same increase over time together with an overall shift toward more multiresistance in all weeks which was due to the common occurrence of SM resistance.

Plate detection method. Because the results obtained by testing 10 colonies per person reflect only the resistances present among the predominant flora, we also used a plate detection method to detect the presence of low numbers of Tmp^r organisms (Table 4). TMP was studied in this manner because it is widely used and was the newest of the agents we studied and because resistance in our previous placebo group in 1980 had been low (3). By the 10-colony method, no participants were shown to be colonized with Tmp^r *E. coli* in week 0, whereas 2 of 13 (15%) were shown by the plate detection method to be colonized (Table 4). By week 1, although only 1 person had Tmp^r *E. coli* by the 10-colony

TABLE 2. Resistance to individual antibiotics

Wk	% of persons[a] with at least one colony resistant to:								
	AP[b]	CM[b]	KM[c]	GM[d]	SM[e]	SU[c]	TC[c]	TMP[e]	TMP-SMX[e]
0	33 (38)	11 (8)	0 (0)	0 (0)	44 (38)	55 (62)	44 (46)	0 (0)	0 (0)
1	11 (8)	33 (15)	0 (8)	0 (0)	55 (38)	88 (85)	44 (38)	11 (8)	22 (15)
2	55 (62)	66 (69)	11 (15)	11 (8)	88 (77)	66 (54)	88 (85)	11 (15)	0 (8)
3	63 (64)	13 (9)	25 (18)	0 (0)	100 (100)	88 (91)	100 (72)	0 (9)	0 (9)
6	57 (55)	43 (44)	29 (33)	0 (0)	86 (89)	100 (100)	86 (89)	57 (55)	57 (55)
7	60 (71)	20 (43)	40 (29)	0 (0)	100 (100)	100 (100)	80 (86)	20 (43)	20 (43)

[a] Percentages are for the participants who took no antibiotics at any time during the study; those in parentheses are for all participants.
[b] Trend for an increasing percentage with resistance was significant, $P < 0.05$, by the Armitage test for trend (7) (determined for all participants).
[c] $P < 0.01$.
[d] Not significant.
[e] $P < 0.001$.

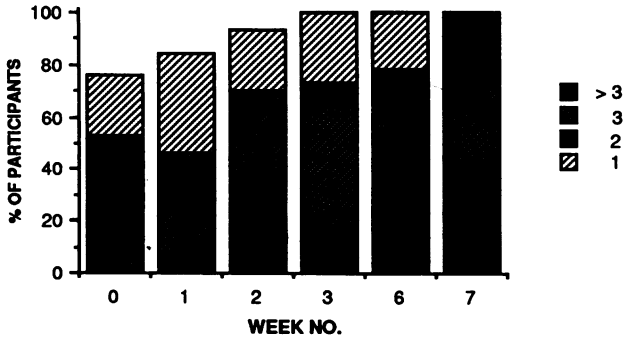

FIG. 1. Resistance to multiple antimicrobial agents among fecal *E. coli* strains. Percentages of participants with *E. coli* resistant to multiple antimicrobial agents increased with time. Bars indicate the presence of *E. coli* strains that are singly or collectively resistant to 1, 2, 3, or >3 agents.

method, 10 of 13 (77%) were colonized by Tmp^r *E. coli* as determined by the plate detection method. By the plate detection method, TMP resistance remained relatively stable during weeks 2, 3, and 6 (in Mexico), with a decline in resistance noted after travelers returned to Houston (week 7). It was not until week 6 that the 10-colony method showed an increase in colonization by Tmp^r *E. coli*. It is of interest that all Tmp^r *E. coli* isolates were resistant to multiple agents, with a minimum of three other resistances (SM, SU, and either AP or TC) and a maximum of six other resistances (resistance to all agents tested except GM).

DISCUSSION

Several settings are well recognized as having high levels of organisms with antibiotic resistance, including animal feedlots, hospital intensive care units, developing countries, and, more recently, day-care centers (2, 3, 5, 9–11, 15–18, 19–22). The problem of resistance is an important one, since it can complicate the choice of therapeutic agents and can create situations in which no effective therapy is known or available locally. For example, shigellae resistant to essentially all oral antibiotics, including nalidixic acid, have been well documented in some developing countries; such organisms severely compromise therapeutic options (9, 16).

Various studies have evaluated resistance within endemic areas, such as our study showing that over 40% of clinical isolates of *E. coli* obtained in 1983 during a study in Chile and Thailand were resistant to TMP-SMX versus only 4 to 8% of concurrent isolates of *E. coli* reported in the United States (11). Little attention has been given, however, to the effect that visiting a developing country has on the emergence of resistance in the flora of the visitor. We have previously shown that 95% of travelers to Guadalajara, Mexico, who were taking TMP or TMP-SMX rapidly acquired TMP-resistant *E. coli* and replaced their TMP-susceptible *E. coli* with TMP-resistant strains (12). This was in marked contrast to results obtained from prophylaxis studies within the United States which had shown little or no development of resistance and presumably indicates that exposure to resistance organisms is more common in Mexico (6). Ørskov et al. showed that fecal *E. coli* of Peace Corps volunteers changed greatly within 10 days of arrival in Morocco, presumably because of organisms present in their diet, but antibiotic resistance was not evaluated (14). Stenderup, Ørskov, and Ørskov reported TC resistance among fecal *E. coli* from 74 volunteers who traveled to Egypt and the Far East (20). These investigators tested up to five colonies per stool sample from volunteers pretravel, during diarrheal illness, and posttravel. Overall, 16% of pretravel samples had *E. coli* resistant to tetracycline; this increased to approximately 85% in diarrheal samples from both placebo and mecillinam (amdinocillin) prophylaxis groups. Approximately 88% of posttravel samples, regardless of whether prophylactic mecillinam was used, had Tc^r *E. coli*; however, approximately half of the placebo group received short courses of mecillinam for therapy, and the resistance of the flora of individuals who received no antimicrobial agent was not specified (20).

In the present study, we used two different methods to evaluate the emergence of resistance in fecal *E. coli*. The plate detection method always detected more TMP resistance than did the 10-colony method. By the plate detection method, TMP resistance was detected in 2 of 13 (15%) participants before their departure from the United States; this is in general agreement with our previous results by a slightly less sensitive method, which showed that 8% of first-year medical students in the United States had TMP-resistant *E. coli* (submitted for publication), and with the study of Levy et al. in 1987, which showed that 7.5% of medical students had TMP-resistant *E. coli* (8). By the end of a week in Mexico, TMP-resistant *E. coli* isolates were detected in 10 (77%) of 13 individuals and remained detectable in the majority of individuals throughout the study period. The results generated by the 10-colony method reflect the predominant flora. With this method, the percentages of persons with resistance to AP, SM, SU, and TC increased markedly by week 2 and remained relatively stable thereafter, while for TMP and TMP-SMX, a marked increase occurred only in the last 2 weeks of the study. There was also a steady and highly significant increase in the resistance score (Table 3) and an increase in the percentage of persons with multiple resistances (Fig. 1).

In analyzing our results, we often separated those individuals who had not consumed antibiotics from those who had received one or more short courses of antibiotics. However, an argument could be made for not doing this. First, the data for the four persons who took short courses of antibiotics do not appear to differ from the data for those who did not take antibiotics (Tables 3 and 4). However, the main reason for not excluding these individuals is that the total group likely represents the real-life situation. Since it is known that approximately 40% of U.S. visitors to Mexico can expect to develop diarrhea, it is reasonable to assume that some of these visitors will take antimicrobial agents (4). Therefore, it

TABLE 4. Detection of trimethoprim-resistant *E. coli*[a]

Wk	No. of persons with TMP-resistant *E. coli*/no. tested (%)[b]		
	No antibiotics	Antibiotics	All
0	2/9 (22)	0/4 (0)	2/13 (15)
1	6/9 (66)	4/4 (100)	10/13 (77)
2	6/9 (66)	3/4 (75)	9/13 (69)
3	5/8 (63)	2/3 (66)	7/11 (64)
6	5/7 (71)	2/2 (100)	7/9 (77)
7	2/5 (40)	2/2 (100)	4/7 (57)

[a] Resistance was detected by inoculating stool specimen directly onto selective media containing 50 μg of TMP per ml; colonies were later identified, and their resistance was confirmed.

[b] Of the 13 individuals in the study, 4 took antibiotics at some time during the study; these 4 were analyzed separately from the 9 who did not take antibiotics during the study.

could be argued that an assessment of the effect of travel to developing countries on the emergence of antibiotic resistance should also include those individuals who become ill and take routine courses of antimicrobial therapy.

In conclusion, this study showed that antibiotic-resistant *E. coli* strains were acquired by visitors to Guadalajara, Mexico, even when the visitors were not taking prophylactic or therapeutic antibiotics. Considering the number of persons who travel to Mexico and other developing countries, travel to developing countries may rival other sources of resistant *E. coli* in the United States. The individual and global risks of acquiring and spreading the resistances acquired in this manner are not known.

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