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Lack of Emergence of Resistant Fecal Flora during Successful Prophylaxis of Traveler's Diarrhea with Norfloxacin

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Norfloxacin, a new quinolone carboxylic acid derivative, was compared with an identical-appearing placebo preparation in a prospective, randomized, double-blind trial for prevention of traveler's diarrhea among 120 U.S. students arriving in Mexico. Prophylaxis was continued for 2 weeks. Diarrhea was defined as four unformed stools in 24 h plus an additional symptom of enteric disease. In the norfloxacin prophylaxis group, 4 of 56 subjects (7%) experienced diarrhea, compared with 36 of 59 subjects (61%) in the placebo group. The difference was significant ($P < 0.0001$). In contrast to our previous experience with use of trimethoprim-sulfamethoxazole to prevent traveler's diarrhea, quantitative stool cultures in the norfloxacin-treated group revealed a significant decline of normal aerobic fecal flora during prophylaxis ($P < 0.0005$). Among stool samples from norfloxacin-treated subjects, 32 of 38 (84%) cultured on day 7 and 34 of 37 (92%) cultured on day 14 had no gram-negative bacilli. After norfloxacin was discontinued, fecal flora returned to pretreatment levels. No gram-negative aerobic flora resistant to norfloxacin were found during weekly quantitative cultures before, during, or after therapy.

Numerous medications have been reported to prevent traveler's diarrhea (5). Recent placebo-controlled studies of doxycycline (14–16), trimethoprim-sulfamethoxazole (TMP-SMX) (4), trimethoprim alone (4), and bicozamycin (6) have shown that these agents effectively prevent traveler's diarrhea. TMP-SMX resistance among enteric bacterial pathogens is becoming more common in several areas of the developing world (13, 20). Also, the development of resistance by fecal flora during drug administration limits the use of TMP-SMX as a prophylactic agent against traveler's diarrhea. (10). Norfloxacin, a new quinolone carboxylic acid derivative similar to nalidixic acid, has a potential benefit over the other available antibiotic agents because of its broad spectrum of activity against most enteric pathogens, including *Shigella* spp., *Salmonella* spp., *Escherichia coli*, *Yersinia enterocolitica*, *Campylobacter jejuni*, *Aeromonas hydrophila*, *Pleisomonas shigelloides*, and *Vibrio parahaemolyticus* (2, 18). In contrast to TMP-SMX, no plasmid-mediated resistance to the quinolone derivatives has been documented (13). The present controlled study, comparing norfloxacin with a placebo for the prevention of traveler's diarrhea, examines the impact of norfloxacin on the composition of the fecal flora and the possible development of bacterial resistance during a 14-day prophylaxis period.

MATERIALS AND METHODS

During the summer of 1984, 120 U.S. students attending summer school as part of the University of Arizona and University of San Diego summer school programs in Guadalajara, Mexico, were prospectively studied. All had arrived in Mexico no more than 3 days prior to being randomly assigned to receive either norfloxacin or an identical-appearing placebo. Norfloxacin was given in a dose of 400 mg once daily for 14 days. Each subject completed a daily diary during the 14-day treatment period and was followed for an additional 7 days posttreatment for the occurrence of diarrhea or adverse reactions to the study medication. A complete blood count, urinalysis, liver function tests, and measurement of blood urea nitrogen and creatinine concentrations were performed initially and at the completion of the study to monitor possible adverse reactions to the medication. Stool specimens were obtained initially and weekly during the study to determine colony counts of aerobic gram-negative bacilli per gram of stool and to test for low-level norfloxacin resistance by plating 100 μl of stool on MacConkey agar with and without 16 μg of norfloxacin per ml. The medications were distributed by a randomized code that was not broken until completion of the study. Any subject experiencing diarrhea between the beginning of drug administration and 48 h after the last dose was declared a prophylaxis failure. Diarrhea was defined as the occurrence of at least four unformed stools in 24 h or three unformed stools in 8 h together with an additional symptom of nausea, vomiting, abdominal pain, or fever. All subjects who developed acute diarrhea discontinued the prophylactic agent and were asked to submit a stool specimen. As previously described (9), these stools were analyzed for the presence of *Entamoeba histolytica*, *Giardia lamblia*, *Salmonella* spp., *Shigella* spp., *Campylobacter* spp., *Aeromonas* spp., and *Pleisomonas* spp. Five *E. coli* isolates were selected from each stool culture for transport on peptone stabs to Houston, Tex. These isolates were later tested for heat-labile and heat-stable enterotoxin activity by the Y-1 adrenal cell assay and the suckling mouse assay, respectively (9). Subjects taking norfloxacin who developed diarrhea during the 21-day study had their stool specimens cultured for *Clostridium difficile* and assayed for *C. difficile* toxin after the frozen stools had been sent to Houston. Antimicrobial susceptibilities of enteropathogens from diarrhea cases were determined by the disk diffusion technique for doxycycline, TMP-SMX, and norfloxacin. Statistical

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TABLE 1. Etiology of traveler's diarrhea during prophylaxis

Pathogen found in stool sample	No. of samples or subjects	
	Placebo group	Norfloxacin group
Enterotoxigenic *E. coli*	18	0
Shigella spp.	3	0
None	9	3
No specimen obtained	6	1

analysis was performed by the chi-squared analysis for clinical data and analysis of variance for comparison of the mean colony counts of aerobic gram-negative bacilli per gram of stool. These tests were done with the Statistical Package for the Social Sciences and Minitab (Pennsylvania State University Release). The study was reviewed and approved by the Committee for the Protection of Human Subjects at The University of Texas Health Science Center at Houston.

RESULTS

Five subjects were excluded from analysis. One subject was excluded after developing diarrhea 1 h after the first dose of medication; he was later found to be in the placebo group. Four additional subjects were excluded for various reasons: three were lost to follow-up, and one was given another antibiotic for an unrelated illness; all four were later found to be in the norfloxacin group.

Diarrhea occurred in 4 of 56 subjects (7%) taking norfloxacin and in 36 of 59 subjects (61%) taking the placebo. This difference was significant ($P < 0.0001$). The protection afforded by norfloxacin (percentage with diarrhea in the placebo group minus percentage with diarrhea in the norfloxacin group divided by the percentage with diarrhea in the placebo group) was 88% in this study. After 14 days of drug administration, the subjects remaining in the trial were followed for another 7 days. Diarrhea occurred in 18% of the subjects in both treatment groups. There was only one adverse reaction to the medicine which was felt to be clearly drug related: one patient developed a generalized skin rash 11 days after beginning norfloxacin, compared with none in the placebo treatment group. The medication was discontinued, and the rash quickly resolved.

The etiology of diarrhea cases occurring during the study is shown in Table 1. The experience in the placebo group is typical of that seen in previous studies (4, 6, 7), with 60% having enterotoxigenic *E. coli* strains, 10% with *Shigella* spp., and 30% having no pathogen detected. In three of four cases of illness in the norfloxacin group, no etiologic agent was detected. In the fourth, a diarrheal stool was not submitted for study. Stools from the three norfloxacin-treated diarrhea cases without a pathogen and from an additional six subjects with illness occurring in the 7 days following prophylaxis for which no etiologic agent was identified were similarly negative for *C. difficile* and its toxin.

TABLE 2. Susceptibility of enteropathogens isolated from subjects with traveler's diarrhea

Isolate	No.	No. (%) susceptible to:		
		Doxycycline	TMP-SMX	Norfloxacin
Enterotoxigenic *E. coli*	18	15 (83)	17 (94)	18 (100)
Shigella spp.	3	0 (0)	2 (67)	2 (67)

The susceptibility patterns of the various enteropathogens obtained from diarrhea cases are shown in Table 2. Three enterotoxigenic *E. coli* isolates were resistant to doxycycline, and one was also resistant to TMP-SMX. None of these strains were resistant to norfloxacin. Of the three *Shigella* isolates, all were resistant to doxycycline, and one strain was also resistant to both TMP-SMX and norfloxacin.

The effect of the study medications on fecal flora during prophylaxis is shown in Fig. 1. Prior to starting the medication, there was no significant difference in the number of aerobic gram-negative bacilli per gram of stool between the two groups. On days 7 and 14, however, there was a significant reduction in the mean number of CFU of aerobic gram-negative bacilli in the norfloxacin group ($P < 0.0005$ by analysis of variance). At day 21, 7 days posttreatment, the gram-negative bacillus count had returned to pretreatment levels. No norfloxacin-resistant aerobic gram-negative bacilli were encountered in either group before, during, or after the study.

Aerobic gram-negative bacilli were present in the stools of all subjects whose samples cultured on day 0 and all placebo-treated subjects whose samples were cultured on days 7, 14, and 21 after therapy was initiated. In the norfloxacin-treated subjects, 32 of 38 samples (84%) cultured on day 7 and 34 of 37 samples (92%) cultured on day 14 had no aerobic gram-negative bacilli detectable. Even 7 days after norfloxacin prophylaxis was completed, 1 of 33 samples (3%) cultured had no aerobic gram-negative bacilli.

DISCUSSION

The 88% rate of protection afforded by norfloxacin compares favorably with rates of 59 to 95% afforded by other antibiotics studied previously in controlled trials (4, 6, 14–17). The lowest rate, 68%, occurred in the group of Peace Corps volunteers taking doxycycline in areas where doxycycline resistance had been documented (16).

Of concern in the use of prophylactic antibiotics for traveler's diarrhea is the encouragement of resistance in enteropathogens and normal flora. This was reported previously by our group in a study of TMP-SMX and TMP alone for prophylaxis of traveler's diarrhea (10). In that study,

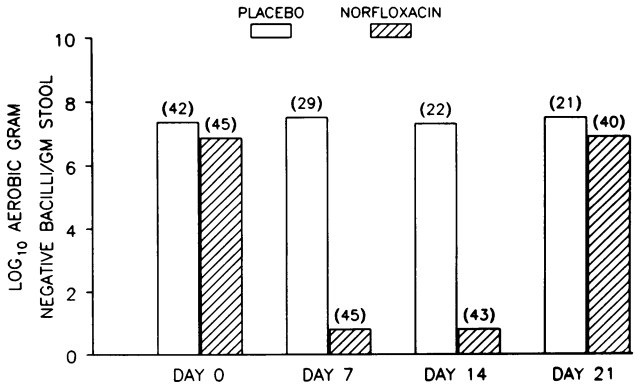

FIG. 1. Aerobic fecal flora during prophylaxis. Norfloxacin significantly decreased the average aerobic gram-negative bacilli counts expressed per gram of stool after 7 and 14 days of treatment ($P < 0.0005$ by analysis of variance). At day 21, 7 days after discontinuation of norfloxacin, aerobic fecal flora returned to normal. Numbers in parentheses show the number of specimens analyzed.

TMP resistance among fecal *E. coli* strains increased during the prophylaxis period. Food in Mexico, which commonly harbors TMP-SMX-resistant coliforms (21), may have been the source of resistant bacteria. The occurrence of resistant flora did not limit the prophylactic effect. In contrast, we did not see the development of resistant flora in the present study. No gram-negative organisms grew in any weekly stool specimens obtained before,during, or after prophylaxis when plated on MacConkey plates containing 16 μg of norflaxacin per ml. One subject in the placebo-treated group developed traveler's diarrhea with a *Shigella* sp. resistant to norfloxacin by disk diffusion testing. Since norfloxacin has not yet become widely used, resistance of fecal flora to the agent may not be prevalent. Another explanation for the lack of development of resistance could be that only chromosome-mediated resistance and not plasmid-mediated resistance to nalidixic acid and its derivatives occurs (13). In serial-passage studies this resistance does not occur as commonly with norfloxacin as it does with nalidixic acid (12). Bicozamycin, an agent for which only chromosomally mediated resistance has been demonstrated, was not commonly associated with the emergence of highly resistant nondiarrheagenic organisms when used for 3 weeks as a preventive against traveler's diarrhea (6). However, bicozamycin is not available commercially, and there are no current plans for its development. Finally, the chance of developing and propagating resistance to norfloxacin may be reduced because of the complete elimination of gram-negative bacilli from the stool in as many as 80% of norfloxacin-treated individuals. This is presumably the result of the high levels of norfloxacin achieved in the stool (3).

Recent studies of doxycycline in areas where resistance to it is common (16, 17) have shown that this agent is not highly effective despite its known concentration in gut secretions. Six of 21 bacterial pathogens (29%) identified in the present study were resistant to doxycycline. As increasing resistance occurs to readily available antimicrobial agents, newer agents such as norfloxacin will have greater value. Although no cases of traveler's diarrhea due to *C. jejuni* were observed in either the placebo or the norfloxacin group, norfloxacin does have in vitro activity against *C. jejuni* and would be expected to be effective in the therapy of campylobacterial entheritis.

Of major concern in the use of these prophylactic agents for traveler's diarrhea is their safety. The only adverse side effect seen in this limited study was skin rash. The rate of skin rash seen here for students taking norfloxacin is comparable to that seen in patients taking TMP-SMX (4). Photosensitivity reactions in patients taking doxycycline who are exposed to the sun during travel occurs at approximately the same frequency. Although serious reactions to norfloxacin have not been reported, our clinical experience with this medication is limited.

While traveler's diarrhea is a self-limiting and nonfatal disease, it occurs in a high percentage of persons traveling to high-risk areas (19). Also, approximately one of five affected persons will be confined to bed, and two of five will have to change their activity level (8). Nevertheless, the occurrence of drug-related side effects when antimicrobial agents are used prophylactically coupled with the finding that antimicrobial agents can be useful in the treatment of traveler's diarrhea argue against their routine use for prophylaxis of traveler's diarrhea (11). We recommend that travelers consult their physician about these issues, and, once the risks and benefits are understood and accepted, elect to use prophylactic antimicrobial agents under certain circumstances: for those taking short trips of a highly important nature or for patients who would be more susceptible to or develop more severe traveler's diarrhea as the result of chronic medical conditions (e.g., peptic ulcer disease, congestive heart failure, diabetes). Antimicrobial prophylaxis should not be used for those remaining in an area of high risk for more than 2 weeks.

In conclusion, norfloxacin is a safe and effective prophylactic agent for traveler's diarrhea when taken for up to 2 weeks during travel. This agent may have advantages over existing prophylactic agents such as doxycycline or TMP-SMX because of the apparent lack of development of resistance during prophylaxis and because of its wide spectrum of activity against diverse bacterial enteropathogens.

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