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Test-of-Cure Stool Cultures for Traveler's Diarrhea

CHARLES D. ERICSSON,[1]* HERBERT L. DuPONT,[1] JOHN J. MATHEWSON,[1] PHILIP C. JOHNSON,[1] F. JAVIER DE LA CABADA,[2] AND JO ANN M. BITSURA[1]

Program in Infectious Diseases and Clinical Microbiology, University of Texas Medical School at Houston, 6431 Fannin Street, Houston, Texas 77030,[1] and Hospital General de Occidente, Guadalajara, Mexico[2]

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Whether enteropathogens were eradicated or persisted in test-of-cure stool cultures from 251 patients with traveler's diarrhea, the durations of diarrhea were similar within the antimicrobial agent-treated (32 versus 33 h) and placebo-treated (82 versus 96 h) groups. Routine test-of-cure stool cultures can be useful for evaluating treatment failures and for assessing asymptomatic carriage of enteropathogens after treatment, but they are not mandated in the design of placebo-controlled antimicrobial treatment trials in traveler's diarrhea when the focus of the trial is clinical efficacy.

Many studies have proven the efficacy of antimicrobial agents in the treatment of traveler's diarrhea (2–6). Study design has often included a repeat stool culture usually performed shortly after the conclusion of therapy to verify eradication of the causal enteropathogen. The rationale for such test-of-cure cultures is that persistence of organisms in stools might explain a lack of symptomatic response in a patient or a potential for relapse of infection that has appeared to respond clinically to therapy. In traveler's diarrhea, clinical success has been seen without eradication of the organism (6). Conversely, in *Campylobacter jejuni* disease eradication of the organism does not always ensure clinical success, at least when treatment is delayed (1, 10); however, both pathogen eradication and clinical success have been achieved with early administration of antibiotics (11). Organisms resistant in vitro to an administered antimicrobial agent have been eradicated from stools, and the patient has responded clinically to the agent, presumably because levels of the antibiotic in stools exceeded the MIC for the organism (9). Finally, since asymptomatic carriage of enteropathogens for a 2-month period can be as high as 37% in developing nations (8), interpretation of a test-of-cure stool culture is further confused by the possibility of simple reacquisition of the pathogen rather than persistence. To understand the utility of test-of-cure stool cultures, we reviewed the results of stool cultures from patients enrolled in trials of antimicrobial agents used to treat traveler's diarrhea and compared these culture results with the clinical outcomes of the patients.

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The population consisted of 452 U.S. adults who attended summer school in Guadalajara, Mexico, and who developed acute diarrhea within 6 weeks of arrival. Diarrhea was defined as passage of four or more unformed stools per 24 h plus an additional symptom of enteric disease, such as nausea, vomiting, abdominal cramps, or temperature elevation. All the subjects were enrolled within 72 h of the onset of diarrhea and treated for 3 to 5 days in previously reported placebo-controlled trials of a variety of antimicrobial agents, including trimethoprim-sulfamethoxazole, trimethoprim alone, bicozamycin, and ciprofloxacin (3–6). Five subjects who were treated with enoxacin (500 mg orally twice a day for 5 days) and not reported elsewhere also were included in the present study. The subjects were asked to submit stool samples for test-of-cure culture on the day after cessation of therapy. If the patient's diarrhea worsened during the treatment period, another stool sample was collected to be analyzed for causal agents before treatment with a known agent was instituted.

Of the 452 subjects enrolled, 167 (37%) (including two enoxacin-treated subjects) had no causal agent identified in their initial stool samples and 34 (7.5%) failed to submit test-of-cure stool samples. A total of 160 (58%) of 277 actively treated patients and 91 (52%) of 175 placebo-treated patients, for a total of 251 (55.5%) of 452 patients, had causal agents identified in stools and submitted test-of-cure samples. The placebo group was smaller than the active group, because in some trials only one arm of three was a placebo control.

Stools were cultured in Guadalajara for the presence of *Salmonella*, *Shigella*, *Campylobacter*, *Aeromonas*, and *Pleisomonas* species and *Escherichia coli* by standard techniques (7). Five colonies of *E. coli*-like organisms were saved on stabs and returned to Houston, Tex., for later testing of heat-stable and heat-labile enterotoxin production, as previously reported (7). Cultures for *Clostridium difficile* and assessments for *C. difficile* cytotoxin in stool were not performed.

The duration of diarrhea was defined as the time from the first dose of medication to passage of the last unformed stool. The mean durations of diarrhea in the treated and placebo groups were compared when the initial pathogen was eradicated, when it persisted, and when a new pathogen was acquired. Pathogen eradications were also compared after the use of different antimicrobial agents and were specifically examined for those 11 subjects who failed to respond clinically to active antimicrobial agent therapy.

Data for *Salmonella* and *Shigella* species were combined, because there were no significant differences in pathogen eradication or clinical outcome. Also, a patient was analyzed in the *Salmonella-Shigella* group regardless of the presence of additional enteropathogens. If either *Salmonella* or *Shigella* species persisted in the test-of-cure culture when both pathogens were initially present, the patient was counted as

* Corresponding author.

TABLE 1. Results of test-of-cure stool cultures and resolution of traveler's diarrhea among subjects treated with antimicrobial agent or placebo

Diarrhea cause	Treatment[a] (no. of subjects)	% of subjects (mean h of diarrhea[b])		
		Causal pathogen		New pathogen acquired
		Persistent	Eradicated	
All known	Antimicrobial agent (160)	10 (33)	90 (32)	4 (7)
	Placebo (91)	32 (96)	68 (82)	13 (68)
Salmonella-Shigella species[c]	Antimicrobial agent (34)	9 (10)	91 (28)	3 (14)
	Placebo (20)	20 (81)	80 (96)	15 (50)
Enterotoxigenic *E. coli*	Antimicrobial agent (103)	2 (80)	98 (32)	5 (17)
	Placebo (53)	28 (109)	72 (74)	19 (87)

[a] Antimicrobial treatment included trimethoprim-sulfamethoxazole, trimethoprim, bicozamycin, ciprofloxacin, and enoxacin.
[b] Hours of diarrhea is the time from the beginning of therapy to passage of the last unformed stool.
[c] When the cause of diarrhea was *Salmonella* or *Shigella* species, the data were lumped (see the text).

having a persisting pathogen. Patients in the enterotoxigenic *E. coli* group had only that organism isolated from enrollment stool samples.

Statistics were performed by using chi-square analysis or the Wilcoxon rank sum test, when appropriate.

Table 1 shows that antimicrobial agent-treated patients had less persistence of the causal enteropathogen than placebo-treated patients. Eradication of enterotoxigenic *E. coli* by antimicrobial agents approached 100%, and eradication was slightly over 90% in the *Salmonella-Shigella* group. On the other hand, persistence of enteropathogens in the placebo-treated group was approximately the same (20 to 28%) in the enterotoxigenic and *Salmonella-Shigella* groups. Overall, a new pathogen was seen in the test-of-cure culture three times more frequently in the placebo-treated group than in the antibiotic-treated group (13 versus 4%).

Table 1 also shows that pathogen eradication and failure to acquire a new pathogen were paralleled by shorter durations of diarrhea in the antimicrobial agent-treated than the placebo-treated groups; however, within the antimicrobial agent-treated group there was no difference in mean durations of diarrhea in subjects whose enteropathogens persisted or were eradicated (33 versus 32 h). Likewise, the durations of *Salmonella-Shigella* diarrhea were similar when the pathogen persisted (10 h) or was eradicated (28 h). Prolonged diarrhea might have occurred when enterotoxigenic *E. coli* persisted (80 h) compared with when it was eradicated (32 h); however, only two subjects (both infected with heat-stable enterotoxin-producing *E. coli*) accounted for the *E. coli* persisters, and the difference (28 versus 80 h) was not significant.

In the antimicrobial agent-treated and placebo-treated subjects in whom new pathogens were isolated, the mean durations of diarrhea (68 and 7 h, respectively) were actually shorter than when the initial pathogen was eradicated (82 and 32 h, respectively). This finding is compatible with the clinical observation that subjects with new pathogens were asymptomatic carriers.

Within the placebo-treated group with enterotoxigenic *E. coli* diarrhea, those subjects who continued to excrete the same pathogen had a longer ($P = 0.008$) duration of diarrhea (109 h) than those in whom the pathogen was eradicated (74 h). There were no significant differences in duration in patients who did or did not excrete *Salmonella* or *Shigella* species in test-of-cure cultures (81 versus 96 h).

Table 2 shows the persistence of the initial enteropathogens in test-of-cure cultures of various antimicrobial treatment groups. The only observation that was significant was that no ciprofloxacin-treated or bicozamycin-treated patients shed the initial pathogen in posttreatment stool specimens compared with 6 of the 28 trimethoprim-treated patients ($P < 0.05$). If the quinolones (ciprofloxacin and enoxacin) were assumed to be equivalent in efficacy, then the finding of one enteropathogen persisting among 27 quinolone-treated patients was still significantly different ($P < 0.05$) from the finding of six enteropathogens persisting among 28 trimethoprim-treated patients.

Finally, of 11 antibiotic-treated patients who failed to respond clinically and who were declared treatment failures, only 1 continued to shed the initial pathogen (*Shigella* species), and none acquired a new enteropathogen.

We believe that routine test-of-cure stool cultures are not mandated in the design of clinical trials of antimicrobial agents for traveler's diarrhea when the aim of the trial is determining the clinical efficacy of the agent in relieving diarrhea. The reason for our belief is that persistence of an initial causal enteropathogen did not predictably correlate with a prolonged course of diarrhea. Since an important expense of conducting trials in traveler's diarrhea is the cost of the stool culture, savings in research cost should be realized by this maneuver.

Ciprofloxacin eradicated significantly more enteropathogens than did trimethoprim (100 versus 79%). If larger numbers had been accumulated in the present study, then ciprofloxacin might have proved to eradicate enteropathogens significantly more frequently than trimethoprim-sulfa-

TABLE 2. Pathogen persistence in test-of-cure cultures by antimicrobial agent used to treat traveler's diarrhea

Treatment (no. of patients)	No. of patients with persistent initial pathogen	% Pathogen eradication
Quinolones (27)	1	96
Ciprofloxacin (24)	0	100
Enoxacin (3)	1	67
Bicozamycin (44)	0	100
Trimethoprim-sulfamethoxazole (61)	8	87
Trimethoprim (28)	6	79

methoxazole. A recent clinical trial, however, failed to demonstrate clinical differences in the efficacies of ciprofloxacin and trimethoprim-sulfamethoxazole in the treatment of traveler's diarrhea (6). No direct comparison of the clinical efficacies of ciprofloxacin and trimethoprim has been done. However, two studies of similar populations showed mean durations of diarrhea of 29, 20, and 81 h after treatment with ciprofloxacin, trimethoprim-sulfamethoxazole, and placebo, respectively (6); and 31, 29, and 93 h after treatment with trimethoprim alone, trimethoprim-sulfamethoxazole, and placebo, respectively (3). These data suggest that differences in pathogen eradication might not accurately predict differences in clinical outcome.

Additional stool cultures are reasonably performed in clinical trials when prolonged shedding of a specific enteropathogen (e.g., *Salmonella* species) is assessed in asymptomatic individuals (carriers) after treatment, but the intent of such cultures should not be to gauge the efficacy of an agent in the relief of clinical symptoms of diarrhea. Finally, although the present study indicated that results frequently may not be particularly helpful, we still subscribe to an additional stool culture when a patient fails to respond to antimicrobial therapy.

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