Microbial Etiology of Travelers' Diarrhea in Mexico, Guatemala, and India: Importance of Enterotoxigenic *Bacteroides fragilis* and *Arcobacter* Species[▽]

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This study examined established enteric pathogens, *Arcobacter* species and enterotoxigenic *Bacteroides fragilis* (ETBF), in 201 U.S. and European travelers with acute diarrhea acquired in Mexico, Guatemala, and India. *Arcobacter butzleri* and ETBF were detected in 8% and 7% of diarrhea cases, respectively.

Etiology studies of travelers' diarrhea (TD) have identified diarrheagenic *Escherichia coli* as the most important cause (>50%) of illness. However, 20 to 40% of subjects remain without a definable cause (5, 6), and antibiotics shorten TD illness without an identified pathogen (2–4), suggesting the presence of occult bacterial enteropathogens.

Bacteroides fragilis strains are part of the normal colonic flora in adults (16). A subclass of *B. fragilis* that secretes a 20-kDa proinflammatory zinc-dependent metalloprotease toxin has been defined as enterotoxigenic *B. fragilis* (ETBF). The strains have been recognized as a cause of acute diarrhea in pediatric and adult populations in regions of endemicity (24).

Arcobacter species are considered emerging food-borne pathogens (9). At present, six species of *Arcobacter* have been characterized, of which *A. butzleri*, *A. cryaerophilus*, *A. skirrowii*, and *A. cibarius* are human- or animal-related pathogens (10). Arcobacters seem not to belong to the normal intestinal flora of humans, but their role in pathogenicity remains unknown (10).

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The population included 201 U.S. and European travelers to Mexico, Guatemala, and India who took part in an ongoing clinical trial. Acute diarrhea was defined as ≥3 unformed stools in 24 h accompanied by one or more gastrointestinal symptoms. Stool samples collected before antibiotic usage were examined for the prevalence of enteric pathogens (12).

Twenty *E. coli* colonies from each stool culture were screened for enterotoxigenic *E. coli* (ETEC) by showing that the organism produced heat-labile enterotoxin (LT) and/or heat-stable enterotoxin (ST) by PCR (15). Five of the 20 isolated *E. coli* colonies were tested for the presence of enteroaggregative *E. coli* (EAEC) by a HEp-2 assay (18).

All stool samples from study sites were cultured for conventional bacterial enteric pathogens, including *Shigella* species, *Salmonella* species, *Vibrio* species, *Campylobacter jejuni*, *Yersinia enterocolitica*, *Aeromonas* species, and *Plesiomonas shigelloides* using previously reported methods (12).

Genomic DNA was extracted from all stools, and PCR was used to target the 16S and 23S rRNA genes of *A. butzleri*, *A. skirrowii*, and *A. cryaerophilus* and the *B. fragilis* toxin (*bft*) gene for ETBF detection (11, 24).

Seventy-six percent of diarrhea stool samples (152/201) tested were ETEC positive, with ST being the primary toxin found for 102 (51%) of subjects (Table 1). The importance of ETEC and specific toxin patterns of the isolates differed by geographic location (Table 1).

Stool samples from a total of 16/201 (8%) patients were positive for *Arcobacter* spp. by PCR detection from diarrhea stools (Table 1). All 16S and 23S rRNA genes of *Arcobacter*-positive stool samples were detected as *A. butzleri*. No other *Arcobacter* spp. were detected. Fifteen subjects had positive ETBF stool samples from 201 diarrhea stool samples (7%), ranging from 13% (6/48) in Goa to 4% (1/25) in Guatemala (Table 1).

At least one enteropathogen was found for 168/201 (84%) patients (Table 1). *A. butzleri* coexisting with ETEC accounted for infection of 13/16 patients. Six of 13 patients with ETEC and *A. butzleri* were detected in Goa, India. For 2/16 patients with *A. butzleri* detected, *Campylobacter* was also identified (data not shown). Seven of 15 patients with TD had both ETBF and ETEC detected. Among the other enteric pathogens identified in the tested specimens, the most frequently

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TABLE 1. Prevalence of enteric pathogens in stools of subjects with travelers' diarrhea acquired in Mexico, Guatemala, or India

Area of isolation	No. of Subjects	Study period(s)	ETEC				EAEC	Arcobacter butzleri	Other Arcobacter spp.	ETEC mixed with A. butzleri	ETBF	ETEC mixed with ETBF	Aeromonas spp.	Campylobacter spp.	Plesiomonas shigelloides	Salmonella spp.	Shigella spp.	Vibrio spp.	Yersinia enterocolitica	No pathogen	Multiple pathogens
			ST	LT	ST/LT	Mixed ETEC															
Goa, India	48	January 2007–April 2007, December 2007–April 2008	29 (60)	3 (6)	4 (8)	2 (4)	3 (6)	7 (15)	0	6 (13)	6 (13)	3 (6)	3 (6)	13 (27)	4 (8)	2 (4)	6 (13)	2	0	3 (6)	29 (60)
Kolkata, India	36	January 2008–August 2008	10 (28)	3 (8)	0	9 (25)	1 (3)	1 (3)	0	0	3 (8)	0	0	4 (11)	0	0	2 (6)	0	0	8 (22)	10 (28)
Antigua, Guatemala	25	June 2007–January 2008	15 (60)	1 (4)	2 (8)	2 (8)	3 (12)	1 (4)	0	0	1 (4)	1 (4)	0	1 (4)	0	1 (4)	0	1 (4)	0	4 (16)	8 (32)
Cuernavaca, Mexico	57	June 2008–August 2008	29 (51)	9 (16)	1 (2)	5 (9)	1 (2)	4 (7)	0	4 (7)	3 (5)	2 (4)	1 (2)	1 (2)	0	0	0	0	0	12 (21)	13 (23)
Guadalajara, Mexico	35	June 2008–August 2008	19 (54)	4 (11)	0	5 (14)	0	3 (9)	0	3 (9)	2 (6)	1 (3)	0	0	0	2 (6)	0	0	0	6 (17)	11 (31)
Total	201		102 (51)	20 (10)	7 (3)	23 (11)	8 (4)	16 (8)	0	13 (6)	15 (7)	7 (3)	4 (2)	19 (9)	4 (2)	5 (2)	8 (4)	3 (1)	0	33 (16)	71 (35)

isolated organisms were *Campylobacter* spp. (9%; 19/201 subjects), followed by *Shigella* spp. (4%; 8/201), *Salmonella* spp. (2%; 5/201), *Aeromonas* spp. or *Plesiomonas* spp. (2%; 4/201), and *Vibrio* spp. (1%; 3/201). *Yersinia enterocolitica* was not identified.

We examined 20 *E. coli* colonies per diarrheal stool sample for ETEC identification, which resulted in a high rate of identification. Clearly, the more colonies studied, the greater likelihood of detecting ETEC (8). To our knowledge, this level of pathogen detection, up to 94% (Goa) of stool samples, has never been reported for studies of TD. As a result, we recommend that more than five *E. coli* colonies be screened when the detection of ETEC is being sought for subjects with TD.

To our knowledge, this is the first study demonstrating *Arcobacter* spp. and ETBF associated with TD. Since its discovery in 1977, *A. butzleri* and *A. cryaerophilus* have been found in stool samples of patients with acute diarrhea (1, 7, 13, 14). A recent study (22) found a 7% *A. butzleri*-positive rate for children with diarrhea, and 55% of *A. butzleri* cases had elevated lactoferrin levels, indicating possible inflammation. Wybo et al. reported the first isolation of *A. skirrowii* from a patient with chronic diarrhea (25). Because the codetection of *A. butzleri* and ETEC was common (13/16 patients), it was not possible to define *A. butzleri* as the causative agent of infection of these patients. ETBF also tended to occur in subjects infected with ETEC isolates, which was seen for 7/15 subjects. Further studies of mixed infections will be needed to determine the contribution of *Arcobacter* and *B. fragilis* to diarrhea cases and to determine the interrelationship between *Arcobacter*, *B. fragilis*, and ETEC in mixed infections.

ETBF is an emerging enteric pathogen associated with diarrheal diseases in children, adults, and animals (17, 19, 24). Sack et al. (20, 21) reported that 12% of isolates from native Americans and 9% of isolates from Bangladeshi children were ETBF positive, compared to 6% of the controls. San Joaquin et al. (23) found a strong association between diarrheal disease and the presence of ETBF in feces of children (isolation rate of 4.8%), whereas *B. fragilis* strains were recovered for 32.1% of children with diarrhea in an urban setting in the United States. In this study, we applied PCR methodology to investigate the presence of ETBF directly in fecal samples. The *B. fragilis* enterotoxin gene (*bft*) was detected from diarrhea stool samples by PCR for 13% of patients with TD in Goa, India. A low recovery rate of ETBF in Guatemala was observed (4%).

PCR-based methods have been described for the direct detection of other bacterial enteropathogens, including *Shigella*, *Salmonella*, and *Campylobacter* species, with the goals of increasing sensitivity and speed of identification. The major problem encountered with PCR-based detection systems for stool samples is that no bacterial isolates are obtained, limiting further studies. Future studies of TD should include conventional pathogens, such as ETEC, EAEC, *Arcobacter*, and ETBF, to better define their geographic importance and potential role in causing TD.

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