Microbial Etiology of Travelers’ Diarrhea in Mexico, Guatemala and India

Importance of Enterotoxigenic Bacteroides fragilis and Arcobacter Species

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Running Title: Enterotoxigenic Bacteroides fragilis and Arcobacter spp

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ABSTRACT
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. *A. butzleri* and ETBF were detected in 8% and 7% of diarrhea cases respectively.

Word Count 42
Etiology studies of travelers’ diarrhea (TD) have identified diarrheagenic *Escherichia coli* are found as the most important causes (>50%) of illness. However, 20-40% 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 endemic regions (24).

*Arcobacter* are considered as emerging foodborne 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).

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

Genomic DNA was extracted from all stools and PCR used to target the 16S and 23S rRNA genes of *A. butzleri*, *A skirrowii*, *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 the primary toxin found in 102 (51%) of subjects (Table). The importance of ETEC and specific toxin patterns of the isolates differed by geographic location (Table).

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

At least one enteropathogen was found in 168/201 (84%) patients (Table). *A. butzleri* coexists with ETEC accounted for 13/16 patients. Six of 13 patients with ETEC and *A. butzleri* were detected in Goa, India. In 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 isolated organism 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) respectively and *Vibrio* (1%, 3/201). *Yersinia enterocolitica* was not identified.
We examined 20 E. coli colonies per diarrheal stool 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) stool samples has never been reported in studies of TD. As a result, we recommend that more than 5 E. coli colonies be screened when ETEC are being sought in 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 detected from stool samples of patients with acute diarrhea (1, 7, 13, 14). A recent study (22) found a 7% A. butzleri positive rate in 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 co-detection with A. butzleri and ETEC was common (13/16 patients), it was not possible to define A. butzleri as the causative agent in these patients. ETBF also tended to occur in subjects infected with an ETEC isolates, seen in 7/15 subjects. Further studies of mixed infections will be needed to determine the contribution of Arcobacter and B. fragilis in 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 in native Americans and 9% in Bangladeshi children were ETBF positive, compared to 6% of the controls. Joaquin et al. (23) found a strong association between diarrheal disease and the presence of ETBF in the feces of children (an isolation rate of 4.8%), whereas B. fragilis strains were recovered in 32.1% of children with diarrhea in an urban setting in the US. In this study, we applied PCR methodology to investigate the presence of ETBF directly in fecal samples.
fragilis enterotoxin gene (bft) was detected from diarrhea stools by PCR in 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 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, ETEC, EAEC, Arcobacter and ETBF to better define their geographic importance and potential role in causing TD.
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Authors disclose no conflict of interest regarding this manuscript.
REFERENCES


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<tr>
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<th>Goa, India</th>
<th>Kolkata, India</th>
<th>Antigua, Guatemala</th>
<th>Cuernavaca, Mexico</th>
<th>Guadalajara, Mexico</th>
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<td>48</td>
<td>36</td>
<td>25</td>
<td>57</td>
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<tr>
<td>ST</td>
<td>29 (60%)</td>
<td>10 (28%)</td>
<td>15 (60%)</td>
<td>29 (51%)</td>
<td>19 (54%)</td>
<td>102 (51%)</td>
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<td>LT</td>
<td>3 (6%)</td>
<td>3 (8%)</td>
<td>1 (4%)</td>
<td>9 (16%)</td>
<td>4 (11%)</td>
<td>20 (10%)</td>
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<td>ST/LT</td>
<td>4 (8%)</td>
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<td>1 (2%)</td>
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<td>Mixed ETEC</td>
<td>2 (4%)</td>
<td>9 (25%)</td>
<td>2 (8%)</td>
<td>5 (9%)</td>
<td>5 (14%)</td>
<td>23 (11%)</td>
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<td><strong>EAEC</strong></td>
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<tr>
<td>Arcobacter butzleri</td>
<td>3 (6%)</td>
<td>1 (3%)</td>
<td>3 (12%)</td>
<td>1 (2%)</td>
<td>0</td>
<td>8 (4%)</td>
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<td>6 (15%)</td>
<td>0</td>
<td>0</td>
<td>4 (7%)</td>
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<td><strong>ETBF</strong></td>
<td>6 (13%)</td>
<td>3 (8%)</td>
<td>1 (4%)</td>
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<td>1 (4%)</td>
<td>2 (4%)</td>
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<td><strong>Aeromonas spp.</strong></td>
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<td>6%</td>
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<td><strong>Plesiomonas shigelloides</strong></td>
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<td><strong>Salmonella spp.</strong></td>
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<td>4%</td>
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<td><strong>Yersinia enterocolitica</strong></td>
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</table>

Total: 195 cases

No. of cases: 3 (6%), 8 (22%), 4 (16%), 12 (21%), 6 (17%), 33 (16%)

No. of cases: 10 (28%), 8 (32%), 13 (23%), 11 (31%), 71 (35%)