Epsilonproteobacteria in Humans, New Zealand

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Using PCR–denaturing gradient gel electrophoresis, we examined 49 fecal samples from healthy volunteers and 128 diarrhea specimens to assess the distribution of Epsilonproteobacteria that might be routinely overlooked. Our results suggest that certain taxa that are not routinely examined for could account for a proportion of diarrhea of previously unknown etiology.

Acute gastrointestinal illness is a major health concern in industrialized countries. In New Zealand, an estimated 4.6 million cases of acute gastrointestinal illness occur every year (1). For many known causes of acute gastrointestinal illness, conventional methods of diagnosis are available; yet, ≈80% of diarrhea cases go undiagnosed (1,2). This lack of data concerning causes of diarrhea hinders the development of intervention strategies.

The class Epsilonproteobacteria is a distinct, diverse bacterial group containing ≥100 taxa (3), including Campylobacter jejuni, recognized as the most frequent bacterial cause of human gastroenteritis worldwide (4,5). Many other epsilonproteobacterial species have been associated with diarrhea, but accurate estimates of the prevalence and role of individual species and proof of a primary pathogenic role have been elusive. Methods commonly used for isolating C. jejuni are not well suited for many other species, and the complex taxonomy of the group makes identification difficult (4). Nevertheless, the body of evidence supporting a causative role for several taxa has grown (5–7). During September 2007–June 2009, we examined fecal samples from healthy volunteers and from patients with diarrhea in New Zealand by using a PCR–denaturing gradient gel electrophoresis (DGGE) method shown to detect and identify Epsilonproteobacteria (8).

The Study

Healthy volunteers were recruited during 2 separate periods in September 2007 and June 2009. The first recruitment period (18 specimens) did not specifically exclude volunteers who had had gastrointestinal disturbances in the 10 days before sampling. The second (31 specimens) were healthy volunteers who had normal bowel habit, no diarrheal disease for ≥6 weeks, no antimicrobial drug therapy for ≥4 weeks, and no medication except for asthma inhalers or antihypertensive or contraceptive medication. Volunteers defecated into a bottle suspended in the lavatory bowl with tissue paper to prevent it falling into the water. The Upper South A Regional Ethics Committee (Christchurch, New Zealand) and the multiethics committee of the Ministry of Health, New Zealand (MEC/08/52/EXP), granted ethics approval for the study.

Diarrhea specimens (submitted without patient details during 2008) were distributed among 3 categories, as follows. First were 32 samples in which no causal agent was found; pathogens were excluded by routine examination with conventional diagnostic techniques for bacteria, parasites, and norovirus at Southern Community Laboratories. Second were 57 samples in which a specific causal agent was not found; samples were examined at the Institute of Environmental Science and Research (ESR, Christchurch, New Zealand) reference laboratory by using conventional methods for a specific pathogen at the request of the submitting laboratory. Third were 39 samples in which a known gastrointestinal pathogen had been detected at ESR.

Samples were refrigerated for 24–48 h before DNA extracts were prepared by using the revised protocol described in the ZR Fecal DNA Kit (Zymo Research, Irvine, CA, USA). Fecal DNA extracts were examined with the PCR-DGGE for Epsilonproteobacteria as described (8). After visualization of the PCR-amplified product, individual DNA bands were excised and then DNA was eluted by diffusion into buffer and reexamined by PCR to obtain partial 16S rDNA amplicons for sequencing. Sequences were edited (primer sequences were removed) and subsequently compared with those in GenBank by using BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi). Comparisons were made during April 2011. Assignment of sequences to a taxon was based on the E (expect) values obtained and on expert opinion of the taxonomic distance between the most likely matches obtained. BLAST matches yielded E-values ranging from 7.13e-62 to 2.26e-124.

Of 177 samples from the healthy volunteers and patients with diarrhea, 159 contained Epsilonproteobacteria, of which 20 contained >1 taxa (Table). C. rectus/showae, C. sputorum, C. upsaliensis, Helicobacter pullorum, and H. pylori/heilmannii/nemestrinae were detected in 11 (8.6%) of the 128 diarrhea samples but not in fecal specimens from...
were all detected in diarrhea specimens but not in specimens containing these organisms were detected. The pathogen differs significantly from in diarrheal sequelea. Even though C. rectus/showae were also detected only in diarrhea samples, 2 specimens also harbored Cryptosporidium spp. In addition, norovirus was detected in the diarrhea sample in which C. sputorum was found.

We detected C. jejuni/coli in 3 samples examined for, but not containing, E. coli O157, which indicates that some cases of campylobacteriosis go undiagnosed. To our surprise, we detected C. jejuni/coli in several fecal samples from healthy volunteers. This detection might represent asymptomatic carriage of C. jejuni/coli, a phenomenon more commonly observed in developing countries where repeated exposure during a prolonged period results in tolerance (11). The high incidence of infection in New Zealand makes this hypothesis credible.

C. concisus was the most frequently encountered species in this study and occurred in participants from both groups. Strains identified as C. concisus with conventional methods might belong to genetically distinct but phenotypically indistinguishable genomospecies differing in their pathogenic potential (12). The PCR-DGGE used here cannot differentiate C. concisus genomospecies; thus strains detected in volunteers and strains found in diarrhea samples might represent distinct genomospecies with different pathogenic potentials.

We detected C. hominis, C. gracilis, and C. ureolyticus in fecal samples of healthy volunteers and patients with diarrhea. C. hominis has long been considered a commensal (13). A molecular study found C. ureolyticus in 83 (23.8%) of 349 Campylobacter spp.–positive diarrhea samples, but no healthy controls were examined (14). Our data suggest these species are unlikely causes of diarrhea.

Our results indicate that certain Epsilonproteobacteria that are not routinely examined for account for a proportion of diarrhea cases of previously unknown etiology. PCR-DGGE is a useful tool to study the prevalence and distribution of these bacteria. C. concisus genomospecies are frequently detected in human disease (5,15; this study); elucidation of their pathogenicity should be considered a public health research issue.
This project was funded by the New Zealand Ministry of Research, Science, and Technology through an ESR-administered Capability Fund project.

Ms Cornelius has worked at ESR since 1994 and currently leads the Public Health Laboratory in the Food Programme at ESR in Christchurch. Her research interests focus on the use of molecular methods for the detection and subtyping of pathogens.

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