**Campylobacter concisus** Pseudo-Outbreak Caused by Improved Culture Conditions

Carlo Casanova, Alexander Schweiger, Niklaus von Steiger, Sara Droz, Jonas Marschall

Clinical Microbiology, Institute for Infectious Diseases, Bern, Switzerland; Department of Infectious Diseases, Bern University Hospital, Bern, Switzerland

An unusual increase in the number of *Campylobacter concisus* isolates found in stool cultures provoked an outbreak investigation at Bern University Hospital. No epidemiological links were found between the cases, and the *Campylobacter* isolates were clonally unrelated. A change in culture conditions to a hydrogen-rich atmosphere enhancing growth of *C. concisus* was deemed responsible for this pseudo-outbreak.

*Campylobacter concisus* is a fastidious *Campylobacter* species whose pathogenic role in human disease has not been established. Isolation of *C. concisus* from samples has been reported in association with periodontal disease, Barrett’s esophagus (1, 2), enteritis, and inflammatory bowel disease (IBD) (3), and the pathogen has been proposed to be linked to certain hepatobiliary and kidney conditions in children (4). High prevalences of *C. concisus* in stool samples were encountered not only in children and adults suffering from diarrhea (detection rate, 0.7% to 49%) but also in healthy controls (detection rate, 0% to 52%) (1, 3). Immunodeficiency (5) and age extremes (6, 7) appear to be determinants of a higher prevalence in stool. Moreover, *C. concisus* might be detected by PCR almost universally in human saliva samples (3). Thus, it is unresolved whether *C. concisus* is merely a commensal bacterium of the human digestive tract or a true pathogen. In light of its genetic variability, both of these may be true (1, 2). In late 2013, a substantial increase in the number of stool cultures testing positive for *C. concisus* was observed at the Bern University Hospital. In order to rule out an outbreak, an epidemiological investigation was conducted.

Bern University Hospital is a 950-bed tertiary care teaching hospital in Switzerland. In the microbiology laboratory, approximately 2,000 stool samples are cultured for enteropathogenic bacteria each year. For *Campylobacter* cultures, clinical stool specimens were inoculated onto Preston agar plates and incubated in a microaerobic atmosphere at 35°C or 42°C for 48 h. Microaerobic conditions were obtained with gas generator packs (CampyGen, Oxoid, United Kingdom), producing a final atmosphere of 5% O2, 8% CO2, 15% H2, and 72% N2 (replacing 76% of the air with an anaerobic gas mixture containing 70% N2, 20% H2, and 10% CO2). Isolates were identified by matrix-assisted laser desorption–ionization time of flight mass spectrometry (MALDI-TOF MS) (Bruker Biotyper; Bruker Daltonics, Bremen, Germany) and sequence analysis using the MicroSeq 500 16S rRNA gene PCR and sequencing kits (Applied Biosystems, Foster City, CA). Genetic relatedness of the isolates was analyzed by repetitive extragenic palindromic PCR (rep-PCR) (8). A case was defined as any patient with *C. concisus* isolated from a stool sample between 2003 and 2013. Retrospective and prospective case finding was performed, including patients who met the case definition during 2013. Incidence data were taken from electronic data on all samples processed at the microbiology laboratory. The laboratory incidence was defined as the number of *C. concisus* identifications divided by the total number of stool cultures processed in the given time period. Epidemiological and clinical data were taken from the hospital’s electronic patient chart (CGM Phoenix; Parametrix Solutions, Lachen, Switzerland), primarily focusing on acquisition mode (nosocomial versus community acquisition). Nosocomial acquisition was defined as a diagnosis >48 h after hospital admission. Patients diagnosed as outpatients with hospitalization within the previous month were considered to have nosocomial *C. concisus* (3, 9). This outbreak investigation was part of the infection prevention mandate and therefore was not subject to review by the ethics committee.

(This work was partially presented as a poster at the 24th European Congress of Clinical Microbiology and Infectious Diseases, Barcelona, Spain, 10 to 13 May 2014.)

In the decade prior to the increase, *C. concisus* was rarely detected in routine stool cultures (average, 1.1 isolates annually). In 2013, *C. concisus* was isolated from stool specimens from 21 individual patients and from an intestinal biopsy specimen from another patient. In all instances, *C. concisus* was the sole organism with pathogenic potential detected. The incidence increased from an average of 0.03% (January 2012 to May 2013) to 1.92% (June to December 2013) (*P* < 0.001, chi-square test; Fig. 1).

The mean age of the 22 patients included in the analysis was 46.7 years (standard deviation, ±25.9 years; range, 3 months to 85 years). Eleven of 22 patients were female. Eight of 22 patients were outpatients. *C. concisus* was detected >48 h after the first admission in 8 of 14 inpatients and more than 48 h into the admission in 3 of 14 patients, during which the diagnosis was made. Two pa-
Patients (3 and 5) were hospitalized on the same ward during the same time period prior to C. concisus detection, with patient 3 being on contact precautions due to diarrhea of unknown etiology. Prior to detection of C. concisus, 3 of 22 patients had a colonoscopy at our hospital and 1 of 22 had one at an external hospital (with intervals of 1, 4, 122, and 140 days prior to diagnosis). Two patients had colonoscopy on the same ward but months apart from each other. In one additional patient, C. concisus was cultured from biopsy material. Putative risk factors for colonization/infection were found in 13 of 22 patients (immunodeficiency, 6 patients [3 with IBD]; extremes of age, 6 patients; extremes of age and immunodeficiency, 1 patient). Seven of 22 cases suffered from either IBD (n = 4) or chronic kidney disease (n = 3), among which 4 of 7 cases were also immunodeficient. Figure 2 summarizes epidemiological data and the results of rep-PCR-based genotyping.

After reviewing the cases, a change in microaerobic culture conditions was identified as the most likely explanation for the putative outbreak. Shortly before the C. concisus incidence started to increase, an automated system for the evacuation and gas replacement of anaerobic jars had been introduced. In contrast to the previously used microaerobic gas generator packs, which did not produce hydrogen, the resulting atmosphere of the new system contained approximately 15% hydrogen. Some Campylobacter species, such as C. concisus, appear to require increased hydrogen concentrations for optimal growth (10). When subculturing five frozen C. concisus isolates (not the original stool samples) from the study period under both culture conditions, only weak or no growth was encountered with the previous methodology (Fig. 3).

In conclusion, a pseudo-outbreak of C. concisus due to a change in laboratory procedures was identified. A pseudo-outbreak at our hospital and 1 of 22 had one at an external hospital (with intervals of 1, 4, 122, and 140 days prior to diagnosis). Two patients had colonoscopy on the same ward but months apart from each other. In one additional patient, C. concisus was cultured from biopsy material. Putative risk factors for colonization/infection were found in 13 of 22 patients (immunodeficiency, 6 patients [3 with IBD]; extremes of age, 6 patients; extremes of age and immunodeficiency, 1 patient). Seven of 22 cases suffered from either IBD (n = 4) or chronic kidney disease (n = 3), among which 4 of 7 cases were also immunodeficient. Figure 2 summarizes epidemiological data and the results of rep-PCR-based genotyping.
break is defined as an episode of increased disease incidence due to enhanced surveillance or other factors but not related to the disease under study (11). Except for one patient, no epidemiological links suggesting nosocomial transmission were found. In addition, genotyping revealed no close relationship between the isolates available for testing. Unfortunately, the isolate of the first— and potential index— case (3) was not available for genotyping. The introduction of a new microaerobic culture system containing a high hydrogen concentration compared to that of conventional microaerobic conditions presumably led to a better recovery of C. concisus from fecal samples. The clinical significance of C. concisus remains unclear to date but may be easier to determine as diagnostic procedures improve and permit the differentiation between pathogenic and nonpathogenic strains.

ACKNOWLEDGMENTS

We thank Regula Tinguely and Andrea Endimiani for rep-PCR analysis.

REFERENCES


**FIG 3** C. concisus isolate subcultured under previous (A, gas generator pack, only a few pinpoint colonies visible [arrow]) and new culture conditions (B, anaerobic jar supplemented with hydrogen) for 3 days at 42°C.