

1 ***Campylobacter concisus* pseudo-outbreak caused by improved culture**  
2 **conditions**

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15 **Abstract**

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

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25     **Text**

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

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41             Bern University Hospital is a 950-bed tertiary care teaching hospital in  
42     Switzerland. In the microbiology laboratory, approximately 2,000 stool samples are  
43     cultured for enteropathogenic bacteria each year. For *Campylobacter* cultures, clinical  
44     stool specimens were inoculated onto Preston agar plates and incubated in a microaerobic  
45     atmosphere at 35°C and 42°C, respectively, for 48 hours. Microaerobic conditions were  
46     obtained with gas generator packs (CampyGen, Oxoid, UK) producing a final atmosphere  
47     of 5% O<sub>2</sub>, 10% CO<sub>2</sub> and 85% N<sub>2</sub>, or with evacuation and gas replacement of anaerobic  
48     jars (TRILAB, Jenny Science, Switzerland) containing approximately 5% O<sub>2</sub>, 8% CO<sub>2</sub>,  
49     15% H<sub>2</sub> and 72% N<sub>2</sub> (replacing 76% of the air with an anaerobic gas mixture containing  
50     70% N<sub>2</sub>, 20% H<sub>2</sub> and 10% CO<sub>2</sub>). Isolates were identified by matrix-assisted laser

51 desorption/ionization time-of-flight mass spectrometry (Bruker Biotyper MALDI-  
52 TOF/MS, Bruker Daltonics, Bremen, Germany) and sequence analysis using the  
53 MicroSeq®500 16S rDNA PCR and Sequencing Kits (Applied Biosystems, Foster City,  
54 CA). Genetic relatedness of isolates was analyzed by repetitive extragenic palindromic  
55 PCR (rep-PCR) (8). Cases were defined as all patients with *C. concisus* isolated from  
56 stool samples between 2003 and 2013. Retrospective and prospective case finding was  
57 performed including patients meeting the case definition during 2013. Incidence data  
58 were taken from electronic data on all samples processed at the microbiology laboratory.  
59 The laboratory incidence was defined as number of *C. concisus* identifications divided by  
60 the total number of stool cultures processed in the given time period. Epidemiological and  
61 clinical data were taken from the hospital's electronic patient chart (CGM Phoenix,  
62 Parametrix Solution, Lachen, Switzerland), primarily focusing on acquisition mode  
63 (nosocomial vs. community-acquired). Nosocomial acquisition was defined as diagnosis  
64 >48 hours after hospital admission. Patients diagnosed as outpatients with hospitalization  
65 within the previous month were considered to have nosocomial *C. concisus* (3, 9). This  
66 outbreak investigation was part of the infection prevention mandate and therefore not  
67 subject to review by the ethics committee.

68 (This work was partially presented as a poster at the 24<sup>th</sup> ECCMID 2014 in Barcelona,  
69 Spain.)

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71 In the decade prior to the increase *C. concisus* was rarely detected in routine stool  
72 cultures (on average 1.1 isolates annually). In 2013 *C. concisus* was isolated from stool  
73 specimens of 21 individual patients and from an intestinal biopsy of another patient. In all  
74 instances, *C. concisus* was the sole organism with pathogenic potential detected. The  
75 incidence increased from an average of 0.03 % (1/2012- 5/2013) to 1.92% (June-  
76 December 2013);  $p < 0.001$ , chi-square test (Fig. 1).

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

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

102 In conclusion, a pseudo-outbreak of *C. concisus* due to a change in laboratory procedures  
103 was identified. A pseudo-outbreak is defined as an episode of increased disease incidence

104 due to enhanced surveillance or other factors but not related to the disease under study  
105 (11). Except for one patient, no epidemiological links suggesting nosocomial  
106 transmission were found. In addition, genotyping revealed no close relationship between  
107 the isolates available for testing. Unfortunately, the isolate of the first – and potential  
108 index - case (#3) was not available for genotyping. The introduction of a new  
109 microaerobic culture system containing a high hydrogen concentration compared to  
110 conventional microaerobic conditions presumably led to a better recovery of *C. concisus*  
111 from fecal samples. The clinical significance of *C. concisus* remains unclear to date but  
112 may be easier to determine as diagnostic procedures improve and permit the  
113 differentiation between pathogenic and non-pathogenic strains.

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127 **Figure legends**

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129 Figure 1: (A) Annual number of clinical samples and patients positive for *C. concisus*  
130 from 2003 to 2013. (B) Absolute numbers (squares) and incidence (solid line) of *C.*  
131 *concisus* isolates from January 2012 to December 2013. The arrow indicates the  
132 introduction of the new culture method.

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134 Figure 2: Results of genotyping and epidemiologic data of all 22 patients diagnosed with  
135 *C. concisus* in stool samples taken in 2013. One strain was isolated from an intestinal  
136 biopsy (patient #17). Patients are numbered in the order of collected culture. A strain (X)  
137 isolated in 2010 was included as unrelated control for typing purposes. NA, not available;  
138 m, male; f, female

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

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