

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% O₂, 10% CO₂ and 85% N₂, or with evacuation and gas replacement of anaerobic
48 jars (TRILAB, Jenny Science, Switzerland) containing approximately 5% O₂, 8% CO₂,
49 15% H₂ and 72% N₂ (replacing 76% of the air with an anaerobic gas mixture containing
50 70% N₂, 20% H₂ and 10% CO₂). 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 24th 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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116 We thank Regula Tinguely and Andrea Endimiani for repPCR analysis.

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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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