Introduction

Campylobacter species are important pathogens in enteric illnesses. The species most commonly associated with human infection are Campylobacter jejuni and Campylobacter coli. Other Campylobacter species are also known to cause human infections (Abbott et al., 2005; Blaser & Allos, 2005; Chua et al., 2007; Vandamme et al., 1989). Many of these non-jejuni, non-coli campylobacters may play a greater role in causing human and animal disease than previously recognized (Blaser & Allos, 2005; Lastovica & Skirrow, 2003).

In the current case report, we describe a case of gastroenteritis caused by Campylobacter concisus. Phenotypic species determination was inconclusive, so the species was determined by sequencing part of the 16S rRNA gene. Furthermore, we discuss the problems encountered in the determination of Campylobacteraceae and the role of C. concisus in human clinical infections.

Case report

A 55-year-old Caucasian male presented to our hospital with complaints of abdominal cramps and frequent watery diarrhoea for 1 week, accompanied by loss of appetite and 5 kg weight loss. His complaints started following chemotherapy. The patient had a history of rectum carcinoma 2 years previously, treated by low anterior resection and temporary colostomy. One year previously, a solitary liver metastasis was treated by hemihepatectomy, followed by chemotherapy in a trial setting. On examination, the patient was moderately ill. His temperature was 37.7 °C, blood pressure was 140/80 mmHg, heart rate was 90 beats min⁻¹ and oxygenation 96 % and there was loss of skin turgor. The abdominal examination was unremarkable besides diffuse abdominal pain. The remainder of the physical examination was normal. The haemoglobin concentration was 7.8 mmol l⁻¹, haematocrit 0.37, white cell count $5.7 \times 10^9$ l⁻¹, thrombocytes $228 \times 10^9$ l⁻¹, C-reactive protein 5 mg l⁻¹, sodium 140 mmol l⁻¹, potassium 3.7 mmol l⁻¹, creatinine 92 μmol l⁻¹ and urea 4.5 mmol l⁻¹. Liver enzymes and bilirubin were within normal limits. Chest X-ray and abdominal X-ray were normal.

The patient was admitted for intravenous rehydration and stool samples were collected. Local recurrence of rectum carcinoma was excluded by sigmoidoscopy. His abdominal cramps were treated with mebeverine 200 mg twice daily and a loperamide scheme was introduced to control the diarrhoea.

Three stool samples were examined in our laboratory. All samples were positive for a spiral-shaped Gram-negative rod, presumptively identified as a Campylobacter species. Based on the findings from the stool culture, the patient received antibiotic therapy with clarithromycin 250 mg twice daily for 7 days, resulting in full recovery from diarrhoeal complaints. In the absence of other causative organisms of diarrhoea and as there was a good response to clarithromycin, we concluded that the isolated Campylobacter strain was the causative micro-organism of the diarrhoea.

Phenotypic species determination using oxidase, catalase, indoxyl acetate and hippurate tests was inconclusive. Serology for Campylobacter did not reveal IgA or IgM reactivity against a glycene extract of C. jejuni (Ang et al., 2007). The species was determined by sequencing part of the 16S rRNA gene. A 500 bp fragment was amplified with the primers of the MicroSeq Microbial Identification system (Hall et al., 2003). Sequencing reactions were performed on purified PCR product with the same

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain VUMC-TY5895 is JN252306.
primers, using the BigDye Terminator v3.1 cycle sequencing kit (Applied Biosystems). The bands were separated on an ABI Prism 3130 Genetic Analyzer (Applied Biosystems). The sequences were edited with the Bionumerics 5.1 software (Applied Maths). The obtained consensus sequence was submitted to the GenBank using the BLASTN tool (http://www.ncbi.nlm.nih.gov/). Highest similarity was found with a number of strains designated Campylobacter curvus from one study (Abbott et al., 2005), and with some C. concisus type or culture collection strains (98.9–99.8 % identity). However, there was less identity with C. curvus type or culture collection strains (<95 %).

To obtain a reliable species determination, we decided to compare our sequence to that of published type strains and reference strains from bacterial culture collections only. Sequences were downloaded from GenBank and edited to equal length. Furthermore, intervening sequences present in some Campylobacter strains were excised from the sequence data. Sequences were aligned and distance matrices were calculated using Jukes–Cantor correction. A phylogenetic analysis was performed using pseudo-rooted neighbour-joining tree building (Fig. 1). Bootstrap values of 500 simulations were determined and indicated in Fig. 1. This was all performed with the Bionumerics 5.1 software. The sequence clearly clustered with the C. concisus strains (bootstrap value 100 %) and not with the C. curvus strains. We therefore concluded that the strain is C. concisus. The sequence of the strain (VUMC-TY5895) was submitted to GenBank and assigned accession number JN252306.

In addition, we tried to identify the strain using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) on a Microflex LT mass spectrometer [Bruker Daltonik; Flex Control Maldi Biotyper version 3.0β; database 3995 main spectra (June 2011)]. The strain was analysed according to the manufacturer’s instructions. Scores <1.7 represent no reliable identification, a score ≥2.0 is considered reliable species identification, and scores between 1.7 and 2.0 represent reliable identification at the genus level. Performing quadruple measurements, MALDI-TOF MS could not reliably identify the strain to species or genus level (maximum score 1.62). The closest match was to a C. curvus strain. However, C. concisus is not included in the database.

**Discussion**

Detection and phenotypic determination of C. concisus is difficult. The most common isolation method used for
detection of Campylobacter species is isolation from selective media incubated under microaerobic conditions. This isolation technique has been developed to increase the sensitivity of detection of C. jejuni and C. coli but may not support the growth of other potentially pathogenic non-jejuni/coli Campylobacter (Blaser & Allos, 2005).

Distinguishing C. concisus from other Campylobacter species can be difficult. During the 1980s and 1990s, several previously un- or misidentified human clinical Campylobacter specimens were reidentified. Most specimens were isolated from patients with gastrointestinal complaints. Identification with various techniques eventually confirmed these strains as C. concisus (Vandamme et al., 1989; On, 1994).

Molecular identification methods may be necessary for identifying certain species because of phenotypic similarity. Molecular methods using PCR or other nucleic acid techniques have been reported (Klena et al., 2004; Maher et al., 2003; Zhang et al., 2009). For reliable species determination, sequenced PCR products should be compared to published genome sequences of type strains or reference strains from bacterial culture collections. As in Fig. 1, a pseudo-rooted tree using an outgroup can be used.

The role of C. concisus in human gastrointestinal disease is uncertain. Van Etterijck et al. (1996) investigated the pathogenic role of C. concisus in gastrointestinal disease in 174 Belgian children with enteritis and 958 controls. No statistically significant difference in isolation rates (13.2% vs 9%; P=0.15) was found. These results are consistent with later findings from Swedish investigators (Engberg et al., 2000), where C. concisus was recovered from 5 of 107 faecal samples from patients with gastrointestinal complaints and 3 of 107 samples from healthy controls. The prevalence of C. concisus infections in patients with enteric disease in a tertiary Danish hospital was quite high, with 110 C. concisus isolates out of 224 Campylobacter isolates (49%) (Aabenhus et al., 2002). In 73% of the samples, C. concisus was the sole pathogen. Most infected patients (71%) were immunocompromised, as was our patient. C. concisus contains at least four distinct but phenotypically indistinguishable genomospecies that might exhibit differences in their spectra of virulence potential (Aabenhus et al., 2005), which may partly explain why so many persons infected by C. concisus do not develop gastrointestinal symptoms.

C. concisus has recently been suggested as a possible aetiologic agent in Crohn’s disease (CD) (Man et al., 2010), where the presence of C. concisus DNA was studied in faecal samples from children with newly diagnosed CD using a PCR assay targeting the 16S rRNA gene. Fifty-six per cent (35/54) of patient samples were positive, a prevalence significantly higher than that in the healthy [33% (11/33); P=0.008] and non-inflammatory bowel disease controls [37% (10/27); P=0.03]. These results are consistent with the earlier findings of Zhang et al. (2009), who also reported significantly higher PCR positivity of C. concisus in children with CD than in controls (51% vs 2%; P<0.0001).

In conclusion, the clinical relevance of C. concisus in gastrointestinal disease has not been determined definitively. In immunocompromised patients, C. concisus may be an opportunistic pathogen causing episodic or chronic diarrhoea. Recent studies indicate that C. concisus is associated with inflammatory bowel disease in children. Additional research is essential in determining the role of C. concisus in gastrointestinal disease.

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References


