Journal of Medical Microbiology (2007), **56**, 1565–1567 DOI 10.1099/jmm.0.47366-0

Case Report

Campylobacter insulaenigrae causing septicaemia and enteritis

Kyra Chua,[1,2] Volker Gürtler,[1] Janet Montgomery,[1] Margaret Fraenkel,[3] Barrie C. Mayall[1] and M. Lindsay Grayson[2,4,5]

Correspondence
M. Lindsay Grayson
Lindsay.Grayson@austin.org.au

[1]Department of Microbiology, Austin Hospital, Austin Health, Heidelberg, VIC 3084, Australia
[2]Department of Infectious Diseases, Austin Hospital, Austin Health, Heidelberg, VIC 3084, Australia
[3]Department of Nephrology, Austin Hospital, Austin Health, Heidelberg, VIC 3084, Australia
[4]Department of Epidemiology and Preventive Medicine, Monash University, Melbourne, Australia
[5]Department of Medicine, University of Melbourne, Melbourne, Australia

Received 27 April 2007
Accepted 6 July 2007

Campylobacter insulaenigrae is a novel species that has been recently only isolated from marine mammals. This is the first report of *C. insulaenigrae* causing enteritis and septicaemia in a patient with end-stage hepatic and renal disease.

Introduction

Campylobacter jejuni and *Campylobacter coli* are well recognized as major causes of acute gastroenteritis worldwide. More recently, other atypical and emerging *Campylobacter* species have been identified. We present a case of gastroenteritis and septicaemia due to *Campylobacter insulaenigrae*, a recently identified *Campylobacter* species previously only isolated from seals and a porpoise (Foster *et al.*, 2004).

Case report

A 60-year-old woman presented with a fever of 39 °C and rigors associated with abdominal pain, diarrhoea and vomiting over a 24 h period. Her medical history included end-stage renal failure requiring renal replacement therapy with haemodialysis secondary to autosomal dominant adult polycystic kidney and liver disease, which also resulted in massive hepatomegaly with previously mild hepatic function impairment. Other co-morbidities included mild bronchiectasis, hypertension and hypertrophic cardiomyopathy. There was no history of significant animal contact, including marine animals.

Physical examination demonstrated a tense, grossly distended abdomen with massive cystic hepatomegaly and mild generalized tenderness. Haematological and biochemical investigations were as follows: haemoglobin 131 g l^{-1} (normal 115–165), leukocytes 13.4×10^9 l^{-1} (normal 4.0–11.0), platelets 235×10^9 l^{-1} (normal 150–400), C-reactive protein 112 mg l^{-1} (normal 1.6–8.7), erythrocyte sedimentation rate 24 mm h^{-1} (normal 7–18), serum creatinine 381 μmol l^{-1} (normal 30–97), urea 7.1 mmol l^{-1} (normal 2.5–7.7), albumin 28 g l^{-1} (normal 36–48), alkaline phosphatase 296 U l^{-1} (normal 32–91), γ-glutamyltransferase 150 U l^{-1} (normal <38), bilirubin 23 μmol l^{-1} (normal <18), alanine transaminase 11 U l^{-1} (normal <34) and INR 1.7 (normal 1.0). Multiple blood cultures were positive (aerobic bottle) after 2 days incubation for a motile, curved Gram-negative bacillus which was presumptively identified as a non-*jejuni Campylobacter* species (see later). Faecal examination demonstrated no white or red cells and growth of routine bowel flora only.

The patient was initially treated with oral ciprofloxacin, but subsequent *in vitro* susceptibility data suggested that the pathogen was resistant to this agent and the patient's treatment was changed to oral azithromycin. During the next 3 days, the patient deteriorated with increasing abdominal pain and distension, increasing leukocytosis [20.1×10^9 l^{-1} (neutrophils 18.1×10^9 l^{-1})] and inflammatory markers (C-reactive protein >380 mg l^{-1}). Computerized tomography of the patient's abdomen revealed a moderate volume of ascites in addition to the known multiple cysts in the liver and kidneys. An ascitic aspirate demonstrated 820 polymorphs μl^{-1}, with no viable bacteria on Gram stain and cultures were sterile. Given the overall clinical deterioration, there was a concern that this may have been related to poor systemic absorption of the oral azithromycin due to drug binding by concomitantly administered calcium carbonate, and the antibiotics were changed to intravenous meropenem and gentamicin. After 1 week, the gentamicin was ceased due to the onset of symptomatic hearing impairment. During the next 11 weeks, the patient continued to receive meropenem and cleared her bacteraemia. However, she remained intermittently febrile (39 °C), hypoglycaemic and hypotensive, requiring occasional inotropic support in the intensive care unit. Ascites of up to 7 l per day continued to

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of *Campylobacter insulaenigrae* reported in this study is EF433401.

accumulate, with magnetic resonance imaging (MRI) suggesting this to be due to portal hypertension and hepatic inferior vena caval compression. Upper and lower gastrointestinal endoscopy failed to demonstrate any evidence of perforation, while neither abdominal MRI nor combined F-18 FDG positive emission tomography–computerized tomography could confirm whether one of the hepatic or renal cysts was infected, although this was suspected clinically. The patient now remains stable while awaiting combined hepatic and renal transplantation, but has been unable to cease meropenem therapy (total duration 8 months) due to episodes of clinical septicaemia whenever this agent is ceased.

Laboratory characterization of the Gram-negative bacillus blood culture isolate was as follows. The isolate was plated on to horse blood agar (HBA; Oxoid), chocolate agar (Oxoid), Campylobacter Selective Media (CSM; Oxoid) and Mueller–Hinton agar (Oxoid) supplemented with 5 % horse blood (MHBA) and incubated at room temperature (25 °C), 37 °C and 42 °C under microaerophilic conditions (CampyGen; Oxoid). The isolate was also plated on to HBA, chocolate and MacConkey agar (Oxoid) and incubated at 37 °C in air supplemented with 5 % CO_2 and on to Wilkins–Chalgren (Oxoid) agar incubated under anaerobic conditions. Growth was present only on chocolate, CSM and MHBA media incubated under microaerophilic conditions at 37 °C and 42 °C. After 48 h of incubation at 42 °C, small, round, translucent colonies measuring 0.64 mm in diameter were evident. The colonies were pleomorphic when incubated at 37 °C with small and large colony variants with identical biochemical characteristics. Gram staining showed curved Gram-negative rods which were 0.2 μm wide and 4.0 μm long. Organisms were motile with a darting motion characteristic of *Campylobacter* species, determined from a wet prep microscopic examination. Isolates were maintained on chocolate agar under microaerophilic conditions. The isolate was oxidase- and catalase-positive but hydrolysis of sodium hippurate and indoxyl acetate was absent. Nitrate was reduced and hydrogen sulphide was not produced in triple-sugar iron agar. There was no growth on 1 % glycine and 2 % NaCl media (On & Holmes, 1991). However, these latter tests were done using organisms which had been previously frozen to –80 °C. The isolate was resistant to cephalothin (30 μg) and nalidixic acid (30 μg) (Oxoid) by disc diffusion. Antibiotic susceptibilities were

Table 1. Etest MICs for *Campylobacter insulaenigrae* isolated from human blood

Antibiotic	MIC (mg l^{-1}) for *C. insulaenigrae*
Erythromycin	0.064
Azithromycin	0.023
Ciprofloxacin	0.75
Tetracycline	0.125
Chloramphenicol	0.25
Trimethoprim–sulfamethoxazole	>32
Ampicillin	0.125
Ceftriaxone	32
Meropenem	0.008
Ertapenem	0.003
Gentamicin	0.38
Tigecycline	0.016
Linezolid	4

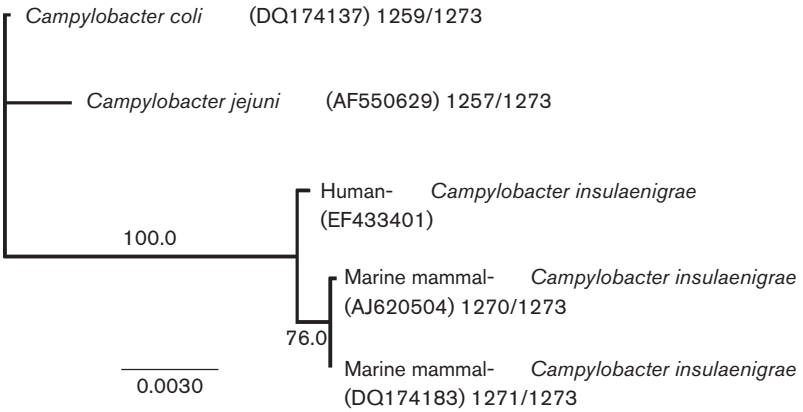

Fig. 1. Phylogenetic relationships derived from 16S rRNA gene sequences for *C. insulaenigrae* (one human and two marine mammal isolates), *C. coli* and *C. jejuni*. Each branch of the phylogenetic tree has been labelled with the species name, GenBank accession number and nucleotide number of the 16S rRNA gene sequence of the species at the respective branch/nucleotide number of the 16S rRNA gene sequence of the human *C. insulaenigrae* isolate. The scale bar shows the number of nucleotide changes along each branch. The numbers on the two lower branches are bootstrap values (number of trees found with this branching pattern in 100 trees analysed). The GenBank accession number for the sequence for the human *C. insulaenigrae* isolate (from this study) is EF433401; the two marine mammal isolates have GenBank accession numbers AJ620504 and DQ174183, respectively, and the GenBank accession numbers for *C. coli* and *C. jejuni* are DQ174137 and AF550629, respectively.

Table 2. Phenotypic characteristics of *C. insulaenigrae* isolated from this patient compared with those of *C. insulaenigrae* reported by Foster *et al.* (2004), *C. coli* and *C. jejuni* subsp. *jejuni*

Symbols: +, 90–100 % of strains positive; d, 21–79 % of strains positive; −, 0–10 % of strains positive. The shading highlights the differences between the human isolate and the Foster *et al.* (2004) isolates. Table modified from Foster *et al.* (2004) with permission.

Characteristic	*C. insulaenigrae* (human isolate)	*C. insulaenigrae* (marine mammal isolates)	*C. coli*	*C. jejuni* subsp. *jejuni*
Catalase	+	+	+	+
Growth at/in:				
25 °C	−	−	−	−
42 °C	+	−	+	+
1 % glycine	−	+	+	+
2 % NaCl	−	−	−	−
Oxidase	+	+	+	+
Nitrate reduction	+	+	+	+
Hydrogen sulfide in triple-sugar iron agar	−	−	d	−
Indoxyl acetate hydrolysis	−	−	+	+
Hippurate hydrolysis	−	−	−	+
Microaerophilic growth	+	+	+	+
Anaerobic growth	−	−	−	−

determined by Etest (AB Biodisk), with results shown in Table 1 using CLSI methods (CLSI, 2006).

To further identify the organism, the 16S rRNA gene of the isolate was amplified by PCR. Total genomic DNA was prepared using the QIAamp DNA Mini kit according to the manufacturer's instructions (Qiagen). Amplification of the 16S rRNA gene was performed with primers and PCR conditions as previously described (Gürtler *et al.*, 1991, 2001). The resulting 1.3 kb product was then purified using the Wizard PCR Preps purification system (Promega) and sequenced by primer walking using the ABI Prism BigDye Terminator v3.0 Ready Reaction Cycle Sequencing kit (Applied Biosystems) with the ABI Prism 3100 Genetic Analyzer. Sequence analysis (Gürtler *et al.*, 2001) demonstrated 98 % sequence similarity with the published sequence for *C. insulaenigrae* (NCTC 12927^T) (Foster *et al.*, 2004); the nucleotide sequence has been assigned the GenBank accession number EF433401. Fig. 1 shows that the 16S rRNA gene sequences from the two isolates from marine mammals differ from each other by 1 nucleotide in 1273 (99.9 % sequence similarity) and the human isolate differs from the marine mammal isolates by 3 nucleotides in 1273 (99.8 % sequence similarity).

Phenotypically, our isolate differed from that described by Foster *et al.* (2004) with growth at 42 °C and no growth in 1 % glycine. A summary of our isolate's phenotypic characteristics compared with those of other members of the genus *Campylobacter* is shown in Table 2.

Discussion

C. insulaenigrae is a recently described species found in marine mammals (seals and porpoise) that is phylogenetically closely related to *C. jejuni*, *C. coli* and *Campylobacter lari* (Foster *et al.*, 2004). Its pathogenic potential is unknown. This is the first report of human disease with this pathogen in a patient who had no contact with marine animals but who was immunocompromised due to hepatic and renal failure and presented with gastroenteritis and septicaemia. The patient's requirement for ongoing antibiotic therapy suggests that one or more of her many hepatic or renal cysts may be infected – an issue that is likely to influence her long-term post-transplant prognosis unless all infected organs can be resected.

Identification of this campylobacter to species level would be difficult if this was based solely on phenotypic testing, and hence relies on 16S rRNA bacterial identification. To the best of our knowledge, this is the first report of clinical human disease caused by *C. insulaenigrae*.

References

CLSI (2006). *Methods for Antimicrobial Dilution and Disk Susceptibility Testing of Infrequently Isolated or Fastidious Bacteria*; Proposed Guideline. M45-P, vol. 25, no. 26, p. 16. Wayne, PA: Clinical and Laboratory Standards Institute.

Foster, G., Holmes, B., Steigerwalt, A. G., Lawson, P. A., Thorne, P., Bryer, D. E., Ross, H. M., Xerry, J., Thompson, P. M. & Collins, M. D. (2004). *Campylobacter insulaenigrae* sp. nov., isolated from marine mammals. *Int J Syst Evol Microbiol* **54**, 2369–2373.

Gürtler, V., Wilson, V. A. & Mayall, B. C. (1991). Classification of medically important clostridia using restriction endonuclease site differences of PCR-amplified 16S rDNA. *J Gen Microbiol* **137**, 2673–2679.

Gürtler, V., Smith, R., Mayall, B. C., Potter-Reinemann, G., Stackebrandt, E. & Kroppenstedt, R. M. (2001). *Nocardia veterana* sp. nov., isolated from human bronchial lavage. *Int J Syst Evol Microbiol* **51**, 933–936.

On, S. L. W. & Holmes, B. (1991). Effect of inoculum size on the phenotypic characterization of *Campylobacter* species. *J Clin Microbiol* **29**, 923–926.