DIAGNOSTIC METHODS

Thermo-tolerant *Campylobacter fetus* bacteraemia identified by 16S ribosomal RNA gene sequencing: an emerging pathogen in immunocompromised patients

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Eight *Campylobacter* isolates that were able to grow at 25°C and 42°C and had the same biochemical profile were isolated from the blood of eight immunocompromised patients. Conventional biochemical tests were unable to determine whether they were isolates of thermo-tolerant *C. fetus*, H₂S-negative *C. hyointestinalis*, or a new *Campylobacter* species. Sequencing of the 16S ribosomal RNA genes showed that all eight isolates had the same nucleotide sequence and this was identical to that of *C. fetus* (GenBank accession no. AF219233). All eight patients had underlying disease and two died despite antibiotic treatment. Because of the ability of *C. fetus* to grow over a wide range of temperatures and a higher incidence of bacteraemia by this organism than *C. jejuni* in the past 5 years in Hong Kong, thermo-tolerant *C. fetus* may be an emerging pathogen in immunocompromised patients in the years to come.

Introduction

Identification of *Campylobacter* spp. is traditionally performed by isolation of the organism and the determination of its gram-staining, cultural and biochemical characteristics. However, these phenotypic methods of bacterial identification have two major drawbacks when they are used for *Campylobacter* speciation. First, some phenotypic characteristics are frequently found to be variable in different *Campylobacter* species. A small percentage of *C. fetus* are able to grow at 42°C, some *C. jejuni* strains are hippurate negative and an increasing percentage of *C. jejuni* strains are resistant to nalidixic acid [1–3]. Second, some characteristics such as production of alkaline phosphatase and growth in glycine 1%, NaCl 1.5% and NaCl 3.5% are highly dependent on the inoculum size and the medium used, and the results of such tests are difficult to interpret [4–6]. Therefore, these tests are

usually not performed in most clinical microbiology laboratories.

Since the discovery of the polymerase chain reaction (PCR) and DNA sequencing, comparison of the gene sequences of bacterial species has shown that the 16S ribosomal (r) RNA gene is highly conserved within a species and among species of the same genus, and hence can be used as the new gold standard for speciation of bacteria. Utilising this principle, bacteria are classified and re-classified into new genera [7, 8], and the genus *Campylobacter* is now classified into 16 different species. Furthermore, bacteria with ambiguous identity because of unusual biochemical profiles can be identified [9]. Recently, the use of this technique has been reported for the identification of bacterial strains with ambiguous biochemical profiles [10–13], species that are rarely encountered clinically [14–16] and a bacterium that cannot be cultured [17], discovery of a novel clinical syndrome [18] and two novel species [19, 20] and characterisation of β -haemolytic Lancefield group G streptococcal bacteraemia [21].

This report describes the application of 16S rRNA gene sequencing in the identification to *Campylobacter*

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isolates from the blood cultures of eight immunocompromised patients.

Materials and methods

Patients, bacterial isolates and microbiological methods

The eight patients in this study were hospitalised at the Queen Mary Hospital in Hong Kong during 1996–2000. All clinical data were collected prospectively as described previously [22]. The BACTEC 9240 blood culture system (Becton Dickinson, MD, USA) was used. All blood culture isolates were identified by standard conventional biochemical methods with *C. fetus* subsp. *fetus* (ATCC 27374), *C. fetus* subsp. *venerealis* (ATCC 19438) and *C. jejuni* subsp. *jejuni* (ATCC 33560) as the control strains [5, 6, 23] and the phenotypic characteristics were compared to those of the known *Campylobacter* species [24–29]. All tests were performed with freshly prepared media on three separate occasions. The MICs of ampicillin, clarithromycin, gentamicin, ciprofloxacin and imipenem were determined by the macrobroth dilution method with Mueller-Hinton broth [30]. The cultures were incubated at 37°C under micro-aerobic conditions. Bacterial growth was examined at 24 h.

Extraction of bacterial DNA for 16S rRNA gene sequencing

Bacterial DNA extraction was modified from a previously published protocol [31]. Briefly, 80 µl of 0.05 M NaOH were added to 20 µl of bacterial cells suspended in distilled water and the mixture was incubated at 60°C for 45 min, followed by addition of 6 µl of 1 M Tris-HCl (pH 7.0) to give a final pH of 8.0. The resultant mixture was diluted 1 in 100 and 5 µl of the diluted extract were used for PCR.

PCR, gel electrophoresis and 16S rRNA gene sequencing

PCR amplification and DNA sequencing of the 16S rRNA gene were performed as described previously [32]. Briefly, distilled water and the PCR master mix containing deoxynucleoside triphosphates (dNTPs), PCR buffer and *Taq* polymerase were first treated by adding 1 U of DNAase I (Pharmacia, Sweden) to 40 µl of distilled water or PCR master mix, incubating the mixture at 25°C for 15 min, and subsequently at 95°C for 10 min to inactivate the DNAase I. The bacterial DNA extract and control were amplified with 0.5 µM primers (LPW57 5'-AGTTTGATCCTGGCTCAG-3' and LPW182 5'-AGTCGCTGATCCACTGTGG-3') (Gibco BRL, Rockville, MD, USA). The PCR mixture (50 µl) contained bacterial DNA, PCR buffer (10 mM Tris-HCl, pH 8.3, 50 mM KCl, 2 mM MgCl₂ and gelatin 0.01%), 200 µM of each dNTP and *Taq* polymerase (Boehringer Mannheim, Germany) 1.0 U. The mixtures

were amplified for 40 cycles of 94°C for 1 min, 55°C for 1 min and 72°C for 2 min, and a final extension at 72°C for 10 min in an automated thermal cycler (Perkin-Elmer Cetus, Gouda, The Netherlands). DNAase I-treated distilled water was used as the negative control. Ten µl of each amplified reaction product, together with molecular size markers (λ DNA *Ava*I digest, Boehringer Mannheim) were electrophoresed in agarose 1.0% w/v gel in Tris-borate-EDTA buffer at 100 V for 1.5 h. The gel was stained with ethidium bromide 0.5 µg/ml for 15 min, rinsed and photographed under UV light.

The PCR product was gel-purified with the QIAquick PCR purification kit (Qiagen, Hilden, Germany). Both strands of the PCR product were sequenced twice with an ABI 310 automated sequencer according to the manufacturer's instructions (Perkin-Elmer, Foster City, CA, USA), with the PCR primers (LPW57 and LPW182) and additional primers designed from the sequencing data of the first round of sequencing reaction (LPW69 5'-AGCACCGGCTAACTCCGT-3' and LPW232 5'-AGTTTTAATCTTGCGA-3'). The sequence of the PCR product was compared with known 16S rRNA gene sequences in the GenBank database by multiple sequence alignment with the CLUSTAL W programme [33]. The phylogenetic tree was constructed by the PileUp method with GrowTree (Genetics Computer Group).

Results

Patient details

The clinical details of the eight patients in this study are summarised in Table 1. The male:female ratio was 1:1. The median age was 64 (range 51–82) years. All eight patients had underlying disease, including liver cirrhosis in five (cases 1, 2, 4, 6 and 7), chronic renal failure in one (case 5), diabetes mellitus in two (cases 3 and 7), intravenous drug abuse in one (case 4) and lymphoma in one (case 2). Six patients had fever or chills on admission (cases 1, 2, 3, 6, 7 and 8), whereas three had abdominal pain (cases 3, 5 and 6) and two had diarrhoea (cases 5 and 8). Two patients had cellulitis (cases 4 and 6). Six patients had neutrophilia (cases 1, 2, 4, 5, 6 and 8) and all had lymphopenia. All eight isolates were isolated from the blood cultures taken from the patients on the day of admission. Two of the eight patients died despite antibiotic treatment (cases 1 and 5).

Phenotypic characteristics of the bacterial isolates

All eight blood culture isolates exhibited the same phenotypic characteristics. They grew on blood agar, chocolate agar and MacConkey agar as grey, flat, irregular colonies of 2 mm diameter after incubation for 48 h at 37°C in micro-aerobic conditions. They

Table 1. Characteristics of eight patients with thermo-tolerant *C. fetus* bacteraemia

Characteristics	Patient no.							
	1	2	3	4	5	6	7	8
Year of presentation	1996	1996	1997	1997	1998	1998	2000	2000
Sex/age (years)	M/64	M/51	F/63	M/66	F/82	M/62	F/75	F/64
Underlying disease	Chronic hepatitis B virus carrier, liver cirrhosis, hepatocellular carcinoma	Lymphoma, chronic hepatitis B virus carrier, liver cirrhosis	Diabetes mellitus, chronic rheumatic heart disease with valvular replacement, congestive heart failure	Alcoholic cirrhosis, intravenous drug use	Chronic renal failure, hypertension, hypercholesterolaemia	Chronic hepatitis B virus carrier, hepatocellular carcinoma	Liver cirrhosis, diabetes mellitus, gallstone	Chronic rheumatic heart disease with valvular replacement, gallstone
Presenting symptoms	Chills and rigor for 1 day	Fever for 1 day	Fever, colicky abdominal pain for 1 day	Right leg cellulitis for 3 days	Abdominal pain for 10 days, watery diarrhoea for 1 day	Fever, abdominal pain, right thigh cellulitis for 1 day	Fever for 1 day	Fever for 10 days, diarrhoea for 4 days
Admission body temperature (°C)	36.0	39.1	38.4	36.8	36.0	39.0	39.2	39.0
Admission WCC (10 ⁹ /L)	16.8	19.5	8.4	20.9	14.6	19.1	6.8	10.3
Admission neutrophil count (10 ⁹ /L)	15.4	18.2	7.3	19.5	13.0	15.6	6.1	9.9
Admission lymphocyte count (10 ⁹ /L)	0.8	0.8	0.5	0.8	0.6	0.6	0.4	0.2
Admission platelet count (10 ⁹ /L)	201	86	115	173	249	167	48	150
Treatment	Ticarcillin-clavulanate	Cefuroxime	Penicillin and gentamicin	Ampicillin and gentamicin	Cefuroxime for 2 days, then 2 doses of ceftazidime before death	Amoxicillin-clavulanate	Amoxicillin-clavulanate	Penicillin and gentamicin
Outcome	Died	Cured	Cured	Cured	Died	Cured	Cured	Cured

Table 2. Phenotypic characteristics of eight blood culture *Campylobacter* isolates and other *Campylobacter* spp.

Organism	Growth at		Catalase	Nitrate reduction	Nitrite reduction	Hippurate hydrolysis	H ₂ S production	Indoxyl acetate hydrolysis	Growth in			Alkaline phosphatase	Susceptibility to	
	25°C	42°C							1% glycine	1.5% NaCl	3.5% NaCl		nalidixic acid	cephalothin
Blood culture isolates	+	+	+	+	—	—	—	—	+	—	—	—	R	S
<i>C. fetus</i> subsp. <i>fetus</i>	+	— ^V	+	+	—	—	—	—	+	V	—	—	R	S
<i>C. fetus</i> subsp. <i>venerealis</i>	+	—	+	+	—	—	—	—	—	—	—	—	R	S
<i>C. coli</i>	—	+	+	+	—	—	—	+	+	—	—	V	S	R
<i>C. concisus</i>	—	+	—	+	+	—	+	—	+	+	—	V	R	S
<i>C. curvus</i>	—	+	—	+	+	—	+	+	+	ND	ND	ND	S	ND
<i>C. gracilis</i>	ND	ND	—	+	+	ND	ND	ND	ND	ND	ND	ND	R	ND
<i>C. helveticus</i>	—	+	—	+	ND	—	—	+	+	ND	ND	—	S	S
<i>C. hyoilei</i>	ND	V	+	+	+	—	+	ND	+	+	—	ND	S	R
<i>C. hyointestinalis</i> subsp. <i>hyointestinalis</i>	V	+	+	+	—	—	+	—	+	—	—	V	R	S
<i>C. jejuni</i> subsp. <i>doylei</i>	—	—	+	—	—	V	—	+	+	—	—	+	S	S
<i>C. jejuni</i> subsp. <i>jejuni</i>	—	+	+	+	—	+	—	+	+	—	—	+	S	R
<i>C. lanienae</i>	—	+	+	+	+	—	—	—	—	ND	ND	+	R	R
<i>C. lari</i>	—	+	+	+	—	—	—	—	+	+	—	—	R	R
<i>C. mucosalis</i>	—	+	—	+	+	—	+	—	+	—	—	V	R	S
<i>C. rectus</i>	—	W	—	+	+	—	+	+	+	ND	ND	ND	S	ND
<i>C. showae</i>	—	+	+	+	+	—	+	+	V	ND	—	—	R	S
<i>C. sputorum</i> bv. <i>Bubulus</i>	—	+	—	+	+	—	+	—	+	+	+	—	R	S
<i>C. sputorum</i> bv. <i>Faecalis</i>	—	+	+	+	+	—	+	—	+	+	+	V	R	S
<i>C. sputorum</i> bv. <i>Sputorum</i>	—	+	—	+	+	—	+	—	+	V	—	ND	S	S
<i>C. upsaliensis</i>	—	+	W/—	+	—	—	—	+	V	—	—	V	S	S

ND, not determined; V, variable; W, weakly positive; R, resistant; S, sensitive.

grew poorly in air with CO₂ 5% at ambient temperature. Microscopic examination of the isolates revealed them all to be gram-negative, slender, curved, seagull-shaped, motile bacilli. They were cytochrome oxidase positive but were unable to metabolise glucose or produce acid from it. They were sensitive to polymyxin B, and negative for arylsulphatase and pyrazinamidase. Thus, they were typical members of the genus *Campylobacter*. The results of further phenotypic tests to distinguish between the blood culture *Campylobacter* isolates and the known *Campylobacter* spp. are summarised in Table 2. All eight blood culture *Campylobacter* isolates were able to grow at 25°C, 37°C and 42°C and in the presence of glycine 1%, but not in the presence of NaCl 1.5% or 3.5%. They were catalase positive, reduced nitrate but not nitrite, did not hydrolyse hippurate or indoxyl acetate, did not produce H₂S or alkaline phosphatase, and were resistant to nalidixic acid but sensitive to cephalothin. The MICs (mg/L) of ampicillin, clarithromycin, gentamicin, ciprofloxacin and imipenem for the eight isolates were 1–4, 1–4, 0.5–1, 0.25–>32 and 0.015–0.03, respectively (Table 3).

16S rRNA gene sequencing

PCR of the 16S rRNA genes of the blood culture *Campylobacter* isolates showed bands at 1449 bp. Sequencing of the 16S rRNA genes showed that all eight isolates had the same nucleotide sequence. There were 11 base differences between those of the isolates and *C. fetus* subsp. *fetus* (GenBank accession no. M65012), 9 base differences between those of the isolates and *C. fetus* subsp. *venerealis* (GenBank accession no. M65011), 16 base differences between those of the isolates and *C. fetus* (GenBank accession no. AF219234), but no base differences between those of the isolates and *C. fetus* (GenBank accession no. AF219233), indicating that the isolates were *C. fetus* (Fig. 1).

Discussion

16S rRNA gene sequencing was used to show that all eight blood culture isolates of *Campylobacter* spp. from eight immunocompromised patients were *C. fetus*.

All eight isolates grew at 42°C, but poorly at 25°C, and did not produce H₂S. Because only a small percentage of *C. fetus* grow at both 25°C and 42°C, phenotypic tests alone were unable to determine whether the isolates were those of a thermo-tolerant *C. fetus*, or an H₂S-negative *C. hyointestinalis*, or a new species of *Campylobacter*.

The data from the present study suggest that these thermo-tolerant *C. fetus* isolates from patients in this locality cause a febrile bacteraemic illness similar to that caused by other *C. fetus* isolates. The median age of the eight patients was 64 years, which is similar to the mean age of 54.1 years reported in a previous study [34]. In three published studies, *C. fetus* bacteraemia was associated with underlying diseases in 41%, 75% and 75% of the patients, respectively [34–36]. In the present study, all eight patients with thermo-tolerant *C. fetus* bacteraemia had major underlying diseases. Similar to previous reports, these patients presented with a febrile illness with or without gastrointestinal symptoms such as diarrhoea and abdominal pain. The association of *C. fetus* bacteraemia with vascular pathology and cellulitis has been well documented [34–36] and was also present in two of the patients in the present study, who had right thigh and leg cellulitis, respectively. Similar to a previous report [35] in which 25% of the patients with *C. fetus* died, the patients in the present study also had significant mortality.

The ability of thermo-tolerant *C. fetus* to grow over a wide range of temperatures can have direct implications for both its natural reservoir and the incidence of bacteraemia. Most *Campylobacter* spp. are adapted to the intestines of warm-blooded animals. Because of their ability to grow at 42°C, *C. jejuni* and *C. lari* strains are particularly adapted to birds, which are probably the major reservoir of these organisms. On the other hand, as most *C. fetus* subsp. *fetus* and all *C. fetus* subsp. *venerealis* are unable to grow at 42°C, they are less commonly found in birds. The main reservoirs for these two species are cattle and sheep. Because thermo-tolerant *C. fetus* isolates can grow at 25°C, 37°C and 42°C, they may be able to adapt to growth not only in cattle and sheep, but also in birds. In a national survey in the UK for 1981–1991, *C. jejuni* accounted for 80% but *C. fetus* for only 9% of the

Table 3. MICs of ampicillin, clarithromycin, gentamicin, ciprofloxacin and imipenem for the thermo-tolerant *C. fetus* isolates from the eight patients

Isolate from patient no.	MICs (mg/L)				
	Ampicillin	Clarithromycin	Gentamicin	Ciprofloxacin	Imipenem
1	1	1	1	0.25	0.015
2	1	1	0.5	0.5	0.03
3	1	2	0.5	0.5	0.015
4	1	1	0.5	0.5	0.015
5	1	2	1	0.5	0.03
6	1	1	0.5	0.25	0.015
7	4	2	0.5	>32	0.03
8	4	4	0.5	2	0.03

Fig. 1. Phylogenetic tree showing the relationship of the eight *C. fetus* isolates to other *Campylobacter* spp. The tree was inferred from 16S rRNA sequence data by the neighbour-joining method. The scale bar indicates the estimated number of substitutions per 100 bases using the Jukes-Cantor correction. Names and accession nos are given as cited in the GenBank database.

Campylobacter bacteraemic isolates [34]. It has been suggested that the rarity of *C. fetus* bacteraemia is due to its predilection for growth at 25°C. In this study and contrary to the previous report, thermo-tolerant *C. fetus* was the predominant *Campylobacter* sp. causing bacteraemia. It accounted for 53% of the *Campylobacter*

bacteraemia in the period 1996–2000, whereas *C. jejuni* subsp. *jejuni*, *C. coli* and *C. lari* accounted for only 33% (five cases), 7% (one) and 7% (one) of the *Campylobacter* bacteraemias, respectively. This predominance of *C. fetus* in causing bacteraemia in the study locality may be due to the ability of thermo-

tolerant isolates to grow at a wider range of temperatures. As a result of this tolerance to a wider range of temperatures, leading to a possible wider range of natural hosts as well as a high incidence of bacteraemia in the past 5 years, thermo-tolerant *C. fetus* may be an emerging pathogen in immunocompromised patients in the years to come.

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References

- Altwegg M, Burnens A, Zollinger-Iten J, Penner JL. Problems in identification of *Campylobacter jejuni* associated with acquisition of resistance to nalidixic acid. *J Clin Microbiol* 1987; **25**: 1807–1808.
- Endtz HP, Ruijs GJ, van Klingeren B, Jansen WH, van der Reyden T, Mouton RP. Quinolone resistance in campylobacter isolated from man and poultry following the introduction of fluoroquinolones in veterinary medicine. *J Antimicrob Chemother* 1991; **27**: 199–208.
- Harvey SM. Hippurate hydrolysis by *Campylobacter fetus*. *J Clin Microbiol* 1980; **11**: 435–437.
- Huysmans MB, Turnidge JD, Williams JH. Evaluation of API Campy in comparison with conventional methods for identification of thermophilic campylobacters. *J Clin Microbiol* 1995; **33**: 3345–3346.
- On SLW, Holmes B. Reproducibility of tolerance tests that are useful in the identification of Campylobacter. *J Clin Microbiol* 1991; **29**: 1785–1788.
- On SLW, Holmes B. Assessment of enzyme detection tests useful in identification of Campylobacter. *J Clin Microbiol* 1992; **30**: 746–749.
- Olsen GJ, Overbeek R, Larsen N et al. The Ribosomal Database Project. *Nucleic Acids Res* 1992; **20** Suppl: 2199–2200.
- Olsen GJ, Woese CR. Ribosomal RNA: a key to phylogeny. *FASEB J* 1993; **7**: 113–123.
- Magee JT, Fox JD, Stubbs SLJ. Cashing in your chips: speculation on the future of diagnostic laboratories in the era of DNA chips. *J Med Microbiol* 2001; **50**: 111–115.
- Woo PCY, Fung AMY, Lau SKP, Yuen K-Y. Identification by 16S ribosomal RNA gene sequencing of *Lactobacillus salivarius* bacteremic cholecystitis. *J Clin Microbiol* 2002; **40**: 265–267.
- Woo PCY, Leung ASP, Leung KW, Yuen KY. Identification of slide coagulase positive, tube-coagulase negative *Staphylococcus aureus* by 16S ribosomal RNA gene sequencing. *J Clin Pathol: Mol Pathol* 2001; **54**: 244–247.
- Woo PCY, Cheung EY, Leung K, Yuen K. Identification by 16S ribosomal RNA gene sequencing of an Enterobacteriaceae species with ambiguous biochemical profile from a renal transplant recipient. *Diagn Microbiol Infect Dis* 2001; **39**: 85–93.
- Woo PCY, Leung PKL, Leung KW, Yuen KY. Identification by 16S ribosomal RNA gene sequencing of an Enterobacteriaceae species from a bone marrow transplant recipient. *J Clin Pathol: Mol Pathol* 2000; **53**: 211–215.
- Lau SK, Woo PC, Teng JL, Leung KW, Yueng KY. Identification by 16S ribosomal RNA gene sequencing of *Arcobacter butzleri* bacteraemia in a patient with acute gangrenous appendicitis. *J Clin Pathol: Mol Pathol* 2002; **55**: 182–185.
- Woo PC, Tsoi HW, Leung KW et al. Identification of *Mycobacterium neoaurum* isolated from a neutropenic patient with catheter-related bacteremia by 16S rRNA sequencing. *J Clin Microbiol* 2000; **38**: 3515–3517.
- Woo PC, Chong KT, Leung K, Que T, Yuen K. Identification of *Arcobacter cryaerophilus* isolated from a traffic accident victim with bacteraemia by 16S ribosomal RNA gene sequencing. *Diagn Microbiol Infect Dis* 2001; **40**: 125–127.
- Cheuk W, Woo PCY, Yuen KY, Yu PH, Chan JKC. Intestinal inflammatory pseudotumour with regional lymph node involvement: identification of a new bacterium as the aetiological agent. *J Pathol* 2000; **192**: 289–292.
- Woo PCY, Li JHC, Tang W-M, Yuen K-Y. Acupuncture mycobacteriosis. *N Engl J Med* 2001; **345**: 842–843.
- Yuen KY, Woo PC, Teng JL, Leung KW, Wong MK, Lau SK. *Laribacter hongkongensis* gen. nov., sp. nov., a novel gram-negative bacterium isolated from a cirrhotic patient with bacteremia and empyema. *J Clin Microbiol* 2001; **39**: 4227–4232.
- Woo PCY, Tam DMW, Leung K-W et al. *Streptococcus sinsensis* sp. nov., a novel species isolated from a patient with infective endocarditis. *J Clin Microbiol* 2002; **40**: 805–810.
- Woo PCY, Fung AMY, Lau SKP, Wong SSY, Yuen K-Y. Group G beta-hemolytic streptococcal bacteremia characterized by 16S ribosomal RNA gene sequencing. *J Clin Microbiol* 2001; **39**: 3147–3155.
- Luk W-K, Wong SSY, Yuen K-YY et al. Inpatient emergencies encountered by an infectious disease consultative service. *Clin Infect Dis* 1998; **26**: 695–701.
- Burnens AP, Nicolet J. Three supplementary diagnostic tests for *Campylobacter* species and related organisms. *J Clin Microbiol* 1993; **31**: 708–710.
- Alderton MR, Korolik V, Coloe PJ, Dewhirst FE, Paster BJ. *Campylobacter hyoilei* sp. nov., associated with porcine proliferative enteritis. *Int J Syst Bacteriol* 1995; **45**: 61–66.
- Etoh Y, Dewhirst FE, Paster BJ, Yamamoto A, Goto N. *Campylobacter showae* sp. nov., isolated from the human oral cavity. *Int J Syst Bacteriol* 1993; **43**: 631–639.
- Logan JM, Burnens A, Linton D, Lawson AJ, Stanley J. *Campylobacter lanienae* sp. nov., a new species isolated from workers in an abattoir. *Int J Syst Evol Microbiol* 2000; **50**: 865–872.
- Stanley J, Burnens AP, Linton D, On SL, Costas M, Owen RJ. *Campylobacter helveticus* sp. nov., a new thermophilic species from domestic animals: characterization, and cloning of a species-specific DNA probe. *J Gen Microbiol* 1992; **138**: 2293–2303.
- Tanner AC, Badger S, Lai CH, Listgarten MA, Visconti RA, Socransky SS. *Wolinella* gen. nov., *Wolinella succinogenes* (*Vibrio succinogenes* Wolin et al.) comb. nov., and description of *Bacteroides gracilis* sp. nov., *Wolinella recta* sp. nov., *Campylobacter concisus* sp. nov., and *Eikenella corrodens* from humans with periodontal disease. *Int J Syst Bacteriol* 1981; **31**: 432–435.
- Vandamme P, De Ley J. Proposal for a new family, *Campylobacteraceae*. *Int J Syst Bacteriol* 1991; **41**: 451–455.
- Murray PR, Baron EJ, Pfaller MA, Tenover FC, Tenover RH (eds). Manual of clinical microbiology. Washington, DC, American Society for Microbiology Press. 1999.
- Woo PC, Lo CY, Lo SK et al. Distinct genotypic distributions of cytomegalovirus (CMV) envelope glycoprotein in bone marrow and renal transplant recipients with CMV disease. *Clin Diagn Lab Immunol* 1997; **4**: 515–518.
- Woo PCY, Fung AMY, Wong SSY, Tsoi H-W, Yuen K-Y. Isolation and characterization of a *Salmonella enterica* serotype Typhi variant and its clinical and public health implications. *J Clin Microbiol* 2001; **39**: 1190–1194.
- Thompson JD, Higgins DG, Gibson TJ. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res* 1994; **22**: 4673–4680.
- Skirrow MB, Jones DM, Sutcliffe E, Benjamin J. *Campylobacter* bacteraemia in England and Wales, 1981–91. *Epidemiol Infect* 1993; **110**: 567–573.
- Francioli P, Herzstein J, Grob JP, Vallotton JJ, Mombelli G, Glauser MP. *Campylobacter fetus* subspecies fetus bacteremia. *Arch Intern Med* 1985; **145**: 289–292.
- Tilse MH, McAlister TV. Isolation of *Campylobacter fetus* from blood cultures. *Med J Aust* 1981; **2**: 337–338.