DIAGNOSTIC METHODS

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

PATRICK C.-Y. WOO, KIT-WAH LEUNG, HOI-WAH TSOI, SAMSON S.-Y. WONG, JADE L.-L. TENG and KWOK-YUNG YUEN

Department of Microbiology, University of Hong Kong, Queen Mary Hospital and HKU-Pasteur Research Center, Hong Kong

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 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
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'-AGTTTTAATCTTGCGA-3' and LPW232 5'-AGTTTTAATCTTGCGA-3') and LPW182 5'-AGTCGCTATTTCCACGTGGG-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 Aval 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'-AGCACCAGCAATCCCGT-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>
<thead>
<tr>
<th>Characteristics</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Underlying disease</td>
<td>Chronic hepatitis B virus carrier, liver cirrhosis, hepatocellular carcinoma</td>
<td>Lymphoma, chronic hepatitis B virus carrier, liver cirrhosis</td>
<td>Diabetes mellitus, chronic rheumatic heart disease with valvular replacement, congestive heart failure</td>
<td>Alcoholic cirrhosis, intravenous drug use</td>
<td>Chronic renal failure, hypertension, hypercholesterolaemia</td>
<td>Chronic hepatitis B virus carrier, hepatocellular carcinoma</td>
<td>Liver cirrhosis, diabetes mellitus, gallstone</td>
<td>Chronic rheumatic heart disease with valvular replacement, gallstone</td>
</tr>
<tr>
<td>Presenting symptoms</td>
<td>Chills and rigor for 1 day</td>
<td>Fever for 1 day</td>
<td>Fever, colicky abdominal pain for 1 day</td>
<td>Right leg cellulitis for 3 days</td>
<td>Abdominal pain for 10 days, watery diarrhoea for 1 day</td>
<td>Fever, abdominal pain, right thigh cellulitis for 1 day</td>
<td>Fever for 1 day</td>
<td>Fever for 10 days, diarrhoea for 4 days</td>
</tr>
<tr>
<td>Admission body temperature (°C)</td>
<td>36.0</td>
<td>39.1</td>
<td>38.4</td>
<td>36.8</td>
<td>36.0</td>
<td>39.0</td>
<td>39.2</td>
<td>39.0</td>
</tr>
<tr>
<td>Admission WCC (10⁹/L)</td>
<td>16.8</td>
<td>19.5</td>
<td>8.4</td>
<td>20.9</td>
<td>14.6</td>
<td>19.1</td>
<td>6.8</td>
<td>10.3</td>
</tr>
<tr>
<td>Admission neutrophil count (10⁹/L)</td>
<td>15.4</td>
<td>18.2</td>
<td>7.3</td>
<td>19.5</td>
<td>13.0</td>
<td>15.6</td>
<td>6.1</td>
<td>9.9</td>
</tr>
<tr>
<td>Admission lymphocyte count (10⁹/L)</td>
<td>0.8</td>
<td>0.8</td>
<td>0.5</td>
<td>0.8</td>
<td>0.6</td>
<td>0.6</td>
<td>0.4</td>
<td>0.2</td>
</tr>
<tr>
<td>Admission platelet count (10⁹/L)</td>
<td>201</td>
<td>86</td>
<td>115</td>
<td>173</td>
<td>249</td>
<td>167</td>
<td>48</td>
<td>150</td>
</tr>
<tr>
<td>Treatment</td>
<td>Ticarcillin-clavulanate</td>
<td>Cefuroxime</td>
<td>Penicillin and gentamicin</td>
<td>Ampicillin and gentamicin</td>
<td>Cefuroxime for 2 days, then 2 doses of cefazidime before death</td>
<td>Penicillin and gentamicin</td>
<td>Amoxicillin-clavulanate</td>
<td>Amoxicillin-clavulanate</td>
</tr>
<tr>
<td>Outcome</td>
<td>Died</td>
<td>Cured</td>
<td>Cured</td>
<td>Cured</td>
<td>Died</td>
<td>Cured</td>
<td>Cured</td>
<td>Cured</td>
</tr>
<tr>
<td>Organism</td>
<td>Growth at</td>
<td>Catalase</td>
<td>Nitrate reduction</td>
<td>Nitrite reduction</td>
<td>H2S production</td>
<td>Alkaline phosphatase</td>
<td>Alkaline nalidixic acid</td>
<td>Alkaline cephalothin</td>
</tr>
<tr>
<td>-------------------------</td>
<td>-----------</td>
<td>----------</td>
<td>-------------------</td>
<td>-------------------</td>
<td>-----------------</td>
<td>---------------------</td>
<td>-------------------------</td>
<td>----------------------</td>
</tr>
<tr>
<td>Blood culture isolates</td>
<td>++</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>C. fetus subsp. fetus</td>
<td>+</td>
<td>++</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>C. fetus subsp. venerealis</td>
<td>++</td>
<td>++</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>C. coli</td>
<td>+</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>C. concisus</td>
<td>+</td>
<td>+</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>C. curvus</td>
<td>+</td>
<td>+</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>C. gracilis</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>C. fetus subsp. venerealis</td>
<td>+</td>
<td>+</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>C. helveticus</td>
<td>+</td>
<td>+</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>C. hyoiles</td>
<td>ND</td>
<td>V</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>C. hyointestinalis subsp.</td>
<td>V</td>
<td>+</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>C. jejuni subsp. doylei</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>C. jejuni subsp. jejunii</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>C. lanienae</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>ND</td>
<td>ND</td>
<td>R</td>
</tr>
<tr>
<td>C. livi</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>R</td>
</tr>
<tr>
<td>C. mucosuolis</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>R</td>
</tr>
<tr>
<td>C. rectus</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>C. showae</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>C. sporumum bv. Bubulus</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>C. sporumum bv. Faecalis</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>C. sporumum bv.</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>C. upsaliensis</td>
<td>+</td>
<td>W/-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>ND, not determined; V, variable; W, weakly positive; R, resistant; S, sensitive.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
grew poorly in air with CO₂ 5% at ambient temperature. Microscopic examination of the isolates revealed that they were 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. venerealis (GenBank accession no. M65011), 16 base differences between those of the isolates and *C. fetus* subsp. venerealis (GenBank accession no. AF219234), but no base differences between those of the isolates and *C. fetus* subsp. venerealis (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 the 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>
<thead>
<tr>
<th>Isolate from patient no.</th>
<th>Ampicillin (mg/L)</th>
<th>Clarithromycin (mg/L)</th>
<th>Gentamicin (mg/L)</th>
<th>Ciprofloxacin (mg/L)</th>
<th>Imipenem (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0.25</td>
<td>0.015</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>1</td>
<td>0.5</td>
<td>0.03</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>2</td>
<td>0.5</td>
<td>0.015</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>1</td>
<td>1</td>
<td>0.5</td>
<td>0.015</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>1</td>
<td>2</td>
<td>0.5</td>
<td>0.03</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>1</td>
<td>1</td>
<td>0.5</td>
<td>0.25</td>
<td>0.015</td>
</tr>
<tr>
<td>7</td>
<td>1</td>
<td>1</td>
<td>0.5</td>
<td>0.25</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>1</td>
<td>2</td>
<td>0.5</td>
<td>&gt;32</td>
<td>0.03</td>
</tr>
<tr>
<td>9</td>
<td>1</td>
<td>4</td>
<td>0.5</td>
<td>0.03</td>
<td></td>
</tr>
</tbody>
</table>

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

---

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

This work was supported in part by the University Development
350 Fund, the University Research Grant Council and the Committee of
351 Research and Conference Grants, University of Hong Kong.

References

1. Altvegg M, Burnens A, Zollerling-Iten J, Penner JL. Problems in
352 identification of Campylobacter jejuni with acquisition of resistance to nalidixic acid. J Clin Microbiol

2. Barr HW, Nicolaeu GS, van Klippenberg B, Jansen WH, van der
354 Reuden T, Mouton RP. Quinolone resistance in campylobacter
355 isolated from man and poultry following the introduction of
356 fluoroquinolones in veterinary medicine. J Antimicrob Che-

3. Harvey SM. Hippurate hydrolysis by Campylobacter fetus. J Clin Microbiol

4. Huymans MB, Turnidge JD, Williams JH. Evaluation of API
359 Campy in comparison with conventional methods for identifi-
360 cation of thermophile campylobacters. J Clin Microbiol

5. On SLW, Holmes B. Reproducibility of tolerance tests that are
362 useful in the identification of Campylobacters. J Clin Microbiol

6. On SLW, Holmes B. Assessment of enzyme detection tests

7. On SLW, Holmes B. Slide coagulase positive, tube-coagulase negative
366 Staphylococcus aureus isolated from a cirrhotic patient with
367 bacteraemia characterized by 16S ribosomal RNA gene sequencing. J Clin Microbiol

8. On SLW, Holmes B. Isolation and characterization of a
369 Enterobacteriaceae species with ambiguous biochemical profile from a renal
370 transplant recipient. Diagn Microbiol Infect Dis 2001; 39:
371 85–93.


373 865–872.

11. Stanley J, Burnens A, Linton D, On SL, Costas M, Owen RJ. Campylo-
374 bacterium larienae sp. nov., a new thermophilic species isolated from domestic animals: characterization, and cloning of a
375 species-specific DNA probe. J Gen Microbiol 1992; 138:
376 2293–2301.

12. Tanner AC, Badger S, Lai CH, Listgarten MA, Visconti RA, Socransky SS. Wolinella recta sp. nov., a novel gram-

13. Yuen KY, Woo PC, Teng JL, Leung KW, Wong MK, Lau SK. Laribacter hongkongensis gen. nov. sp. nov., a novel gram-


17. Cheuk W, Woo PCY, Yuen KY, Yu PH, Chan JKC. Infectious

18. Woo PCY, Li JHC, Tang W-M, Yuen KY, Acupuncture

19. Yuen KY, Woo PC, Teng JL, Leung KW, Wong MK, Lau SK. Laribacter hongkongensis gen. nov. sp. nov., a novel gram-

20. Murray PR, Baron EJ, Pfaller MA, Tenover FC, Y olken RH


22. Tanner AC, Badger S, Lai CH, Listgarten MA, Visconti RA, Socransky SS. Wolinella recta sp. nov., a novel gram-

23. Murray PR, Baron EJ, Pfaller MA, Tenover FC, Y olken RH


27. Stanley J, Burnens A, Linton D, On SL, Costas M, Owen RJ. Campylo-

28. Tanner AC, Badger S, Lai CH, Listgarten MA, Visconti RA, Socransky SS. Wolinella recta sp. nov., a novel gram-

29. Murray PR, Baron EJ, Pfaller MA, Tenover FC, Y olken RH

30. Murray PR, Baron EJ, Pfaller MA, Tenover FC, Y olken RH


32. On SLW, Holmes B. Evaluation of enzyme detection tests
33. useful in identification of Campylobacter jejuni. J Clin Microbiol
34. 1995; 33: 3345–3346.

35. On SLW, Holmes B. Isolation and characterization of a
36. Enterobacteriaceae species with ambiguous biochemical profile from a renal
37. transplant recipient. Diagn Microbiol Infect Dis 2001; 39:
38. 85–93.

39. On SLW, Holmes B. Identification by 16S ribosomal RNA gene sequencing of a new bacterium as the aetiological

41. Skirrow MB, Jones DM, Surtcliffe E, Benjamin J. Campylo-
