Campylobacter fetus subspecies fetus spondylodiscitis

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Campylobacter spp. are common causes of gastrointestinal infections. Campylobacter fetus is a much rarer pathogen in humans, and usually causes bacteraemia and systemic complications in patients with predisposing conditions. We report a case of spondylodiscitis caused by C. fetus subsp. fetus as revealed by vertebral biopsy culture. This identification was confirmed by sequencing the 16S rRNA gene and by phylogenetic analysis. Treatment consisted of 6 weeks antimicrobial therapy combined with a strict initial immobilization, followed by a re-education program. The patient’s recovery was uneventful.

Case report

A 91-year-old woman, suffering from pain in the left hip and in the lumbar region, had a fever 2 weeks before hospitalization. Radiographs of the lumbar spine revealed only arthritic lesions. She was given a monodose of 400 mg ofloxacin because of a possible urinary tract infection. She was admitted to the University Hospital Centre (2 Boulevard Tonnellé, 37044 Tours Cedex 9, France) because of a persistent fever (38.2 °C) and lower back pain. Her blood pressure was 140/70 mmHg, her absolute polymorphonuclear count was 10 900 cells mm−3, she had a C-reactive protein level of 111.2 mg l−1 and a creatinine clearance rate of 35 ml min−1.

An initial lumbar computed tomography (CT) scan showed an abnormal enhancement at the L2–L3 and L3–L4 disc space level, and in the left psoas muscle after contrast injection (Fig. 1), suggestive of a spondylodiscitis. Magnetic resonance imaging was contra-indicated due to the presence of a pacemaker.

A CT-guided disc vertebral biopsy with a posterolateral approach was performed. Three discitis samples were sent to the microbiology department of the University Hospital Centre. Samples were also sent to the mycology department and to the cytology department of the University Hospital Centre in accordance with French guidelines (SPILF, 2007).

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The patient was placed in strict dorsal decubitus, and an empiric broad-spectrum antibiotic therapy using ofloxacin (400 mg twice daily) plus rifampicin [100 mg (kg body weight)−1 twice daily] was initiated (for the first week). The patient was afebrile 24 h later. Blood cultures (aerobic and anaerobic vials) remained negative (Bactec 9240; BD).

Culture of two of the three disc samples and fluid aspirations produced grey-white non-haemolytic colonies after 48 h incubation on 5% blood Columbia agar (bioMérieux) at 37 °C under aerobic conditions. The growth on trypticase soy agar supplemented with 5% horse blood and on chocolate agar plus PolyViteX (bioMérieux) was very weak. Microscopic examination of these colonies after Gram staining revealed curved Gram-negative rods, and both catalase and oxidase reactions were positive. A Campylobacter species was suspected and colonies were subcultured under microaerophilic conditions (10% CO2, 5% O2, 85% N2) at 37 and 42 °C. Bacteria grew only at 37 °C. Hydrolysis of sodium hippurate was negative. The pattern of susceptibility to antibiotics was resistance to nalidixic acid (30 mg disc) and susceptibility to cefalotin (30 mg disk). These phenotypic findings suggested Campylobacter fetus (Penner, 1988; Pigrau et al., 1997). Identification was confirmed by PCR of the 16S rRNA gene followed by sequencing.

DNA was extracted with an Invisorb spin tissue kit (Invitek). PCR was performed with a 10 µl DNA sample in a mixture containing 0.5 µM primers U115 5′-CCAG-CAGCCGGGTAACTACG-3′ and U215 5′-ATCGGTTACGACTCTCC-3′, 200 µM dNTPs (Boehringer), 2.5 mM MgCl2 (Applied Biosystems) and 2.5 U AmpliTaq DNA (Applied Biosystems) in a final volume of 50 µl (Lu

Abbreviations: CT, computed tomography; SPILF, Société de Pathologie Infectieuse de Langue Française.
et al., 2000). Sequencing was performed using a BigDye terminator v 3.1 cycle sequencing kit (Applied Biosystems). Sequencing analysis 5.1.1 software (Applied Biosystems) was used to analyse the DNA sequence. BLAST software was used to compare the DNA sequence with sequences published in the National Center for Biotechnology Information database. The best identification, according to BLAST analysis, was \textit{C. fetus} (two strains AB301966 and AB301967) with 97% identity for the 434 bases tested. Phylogenetic analysis of the partial nucleotide sequence performed with BIBI software (http://umr5558-sud-str1.univ-lyon1.fr/lebibibi/lebibibi.cgi) confirmed the identification.

\textit{In vitro} susceptibility tests using the Etest method (AB Biodisk) on Mueller–Hinton agar (bioMérieux) supplemented with 5% horse blood, showed susceptibility to ampicillin and levofloxacin, and resistance to erythromycin and ciprofloxacin (Table 1). Previous patterns of susceptibility to antibiotics determined with \textit{C. fetus} isolates showed that resistance to erythromycin was rare (0 to 2.2\% of 114 isolates in France in 2007, 0 of 105 isolates in France in 2008) (King et al., 2008).

Pathological examination of disc samples showed no specific inflammatory reaction and no tumour cells were found. No organisms were seen on Gram staining, Ziehl–Nielsen staining and mycobacterial culture on Löwenstein Jensen medium (Bio-Rad) and in mycobacterial vials (BacT/ALERT; bioMérieux) were performed on disc samples. No acid-fast bacilli were observed and cultures remain negative after 3 months of incubation.

Following bacterial identification and antibiogram results, the antibiotic therapy was changed to oral amoxicillin, 6 g
spondylodiscitis is very rare (Bachmeyer et al., 1997), almost all patients (93 %) had an underlying disease such as cirrhosis of the liver, immunosuppression or neoplasia. The exploratory tests for digestive neoplasia (such as colonoscopy) were not performed in this case but clinical findings did not support neoplasia or immune dysfunction. The case of vertebral osteomyelitis caused by *C. fetus* reported by Yamashita et al. (1999) also occurred in an elderly man with no predisposing conditions.

As with other Gram-negative organisms that colonize mucosal surfaces, the high serum resistance of *C. fetus* strains is related to the ability to cause bacteraemia (Blaser, 1998; Chuman et al., 2003; Pigrau et al., 1997). Various systemic complications may occur, such as meningitis and endocarditis. *C. fetus* endocarditis has been reported in less than 30 cases (Brouqui & Raoult, 2001; Farrugia et al., 1994).

In this case, the patient received ofloxacin for 7 days, which was stopped 2 weeks before microbiological samples were taken. Moreover, blood cultures were negative even though the Bactec system is recognized to allow growth of *Campylobacter*. There was no evidence of infectious endocarditis with the self-limiting transthoracic echocardiography.

Identification of the pathogen was obtained by microbiological examination of disc samples and confirmed by molecular methods. The sensitivity of standard microbiological methods for identifying the cause of infectious spondylodiscitis microbiological cultures ranges from 50 to 80 % (Bontoux et al., 1992; Grammatico et al., 2008; Lecouvet et al., 2004; Torda et al., 1995). Isolation of *C. fetus* is rare in such samples (Bachmeyer et al., 1992; Yamashita et al., 1999). The French National Reference Centre for Campylobacters and Helicobacters (Centre National de Référence des Campylobacters et Helicobacters) reported in 2008 that *C. fetus* represented 3.2 % of 3481 strains isolated, mainly from blood cultures (55.2 %) (King et al., 2008).

In our case, the identification of a *C. fetus* subspecies was confirmed by amplification of the 16S rRNA gene by PCR followed by DNA sequencing. Furthermore, some data

### Discussion

*C. fetus* spondylodiscitis is very rare (Bachmeyer et al., 1992; Mathieu et al., 1991; Yamashita et al., 1999). Therapy in the reported cases included a combination of doxycycline and erythromycin for 3 months or alternate oral administration of each antibiotic for 5 months (Mathieu et al., 1991; Yamashita et al., 1999). There are no international recommendations for the management of lumbar spondylodiscitis. A 10 year retrospective study by Roblot et al. (2007) suggested that antibiotic therapy of vertebral osteomyelitis could be safely shortened to 6 weeks without enhancing the risk of relapse. In the case presented here, the patient had 6 weeks of treatment, with 5 weeks of amoxicillin oral monotherapy, combined with an initial strict dorsal decubitus for 3 weeks, followed by re-education. This treatment led to an uneventful recovery. Although 4–6 weeks is the minimum acceptable time for antimicrobial therapy, a much longer period of treatment may be required to cure individual patients (SPIILF, 2007). Reviews of pyogenic vertebral osteomyelitis found that parenteral antibiotic therapy of less than 4 weeks resulted in therapeutic failure more often than therapy for more than 4 weeks did (McHenry et al., 2002).

*C. fetus* subs. *fetus* infections and especially bacteraemia usually occur in immunocompromised or debilitated elderly patients (Jansen et al., 2008). In the 58 cases of bacteraemia due to *Campylobacter species* reported by Pigrau et al. (1997), almost all patients (93 %) had an underlying disease such as cirrhosis of the liver, immunosuppression or neoplasia. The exploratory tests for digestive neoplasia (such as colonoscopy) were not performed in this case but clinical findings did not support neoplasia or immune dysfunction. The case of vertebral osteomyelitis caused by *C. fetus* reported by Yamashita et al. (1999) also occurred in an elderly man with no predisposing conditions.

### Table 1. MIC determined by Etest method (AB Biodisk)

<table>
<thead>
<tr>
<th>Antimicrobial agent</th>
<th>MIC against strain (µg ml⁻¹)</th>
<th>Breakpoint (µg ml⁻¹)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ampicillin</td>
<td>4</td>
<td>≤ 4</td>
</tr>
<tr>
<td>Ampicillin/ clavulanate</td>
<td>3</td>
<td>≤ 4</td>
</tr>
<tr>
<td>Cefalotin</td>
<td>3</td>
<td>≤ 8</td>
</tr>
<tr>
<td>Imipenem</td>
<td>0.047</td>
<td>≤ 2</td>
</tr>
<tr>
<td>Ertapenem</td>
<td>0.75</td>
<td>≤ 2</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>0.125</td>
<td>≤ 2</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>6</td>
<td>≤ 1</td>
</tr>
<tr>
<td>Levofloxacin</td>
<td>1</td>
<td>≤ 1</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>2</td>
<td>≤ 0.5</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>0.01</td>
<td>≤ 4</td>
</tr>
</tbody>
</table>

*Breakpoints for Gram-negative bacteria were used for imipenem, ertapenem and levofloxacin, and data for *Campylobacter* spp. were used for other antibiotics [French Society for Microbiology recommendations, 2009 (http://www.sfm.asso.fr)].

per day, for 5 weeks; the total antibiotic therapy being for 6 weeks. Strict dorsal decubitus was maintained for 3 weeks followed by progressive ‘reverticalization’ with a lumbar sacral corset. At the 3 and 6 month follow-up stages, the patient remained well, with no fever or back pain, and no inflammatory biological syndrome.

Although the patient had no intestinal symptoms, the portal of entry of *C. fetus* subs. *fetus* was probably the gastrointestinal tract. The patient had no history of gastrointestinal pathology. Stool cultures before specific antibiotic therapy remained negative for *Campylobacter* species, and colonoscopy was not performed because of underlying cardiac diseases such as atrial fibrillation, ischaemic cardiopathy and hypertension in this elderly patient. Despite negative blood cultures, transthoracic echocardiography was performed and no pacemaker-related infectious endocarditis was detected. A transoesophageal echocardiography was not performed, due to the age of the patient.
highlight the sensitivity and specificity of DNA-based diagnostic methods and their contribution to the identification of pathogens in spondylodiscitis. In the 19 patients with spondylodiscitis reported by Lecouvet et al. (2004) the use of molecular methods in addition to microbiological techniques increased the identification rate of bacterial agents from 74% (14 of 19 patients) to 100% (19 of 19 patients). DNA-based methods are highly sensitive and can complement classical microbiological methods to optimize the identification of the causative agents of infectious spondylodiscitis.

This is, to the best of our knowledge, the fourth case of a C. fetus spondylodiscitis in a host with no apparent immunosuppression except for her advanced age. The diagnosis was based on discoterebral biopsy, and the bacterial identification was confirmed by PCR and phylogenetic analysis. Improvements in the detection of C. fetus and related microaerophiles by clinical microbiologists will help us appreciate the true scale of this problem. Amplification-based analysis of bacterial DNA should overcome some of the limitations of classical microbiological methods (Lecouvet et al., 2004; Schulze et al., 2006).

References