Aeromonas veronii biovar sobria bacteraemia with septic arthritis confirmed by 16S rDNA PCR in an immunocompetent adult

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An 81-year-old man developed septic arthritis and bacteraemia with Aeromonas veronii biovar sobria. Cultures of the joint wash-out fluid were negative; however, DNA matching that of Aeromonas veronii was identified by 16S rDNA PCR. The patient was successfully treated with a 4 week course of ciprofloxacin. No recognized risk factors were found.

Introduction
Aeromonas veronii biovar sobria is one of 13 Aeromonas species described that are Gram-negative, facultatively anaerobic bacteria. Amongst these it is the motile species (A. veronii, Aeromonas hydrophila, Aeromonas caviae, Aeromonas jandaei, Aeromonas schubertii) that are potentially pathogenic to humans (Janda et al., 1995). Aeromonas species have a worldwide distribution and have been isolated from freshwater, marine animals, soil and nonfaecal organic materials (Davis et al., 1978). Clinical presentations include gastroenteritis, wound infections, biliary tract infections, pneumonia, meningitis, septic arthritis or septicaemia without an obvious focus of infection. A. veronii biovar sobria is a rare cause of bacteraemia, with several studies indicating that this isolate may be of particular clinical significance because it is enterotoxin producing (Turnbull et al., 1984); different antimicrobial susceptibilities have been noted (Motyl et al., 1985; Travis et al., 1986) and it may be more pathogenic than other Aeromonas species (Daily et al., 1981). This is believed to be the first report where 16S rDNA PCR has been used to identify this organism in a patient with septic arthritis and bacteraemia.

Case report
An 81-year-old Caucasian man was admitted to hospital with a 1 week history of progressive pain and immobility of the right shoulder joint. There had been no history of trauma. He lived alone, had no pets, no history of travel or water contact, and rarely went outdoors. He had a past medical history of poliomyelitis at the age of 4 years with residual paralysis of his left leg, axillary artery thrombosis of both arms in 1993 and a basal cell carcinoma of the scalp that had been successfully excised in 1995. On examination he was febrile at 38·3°C with focal tenderness over the right glenohumeral joint. Blood tests demonstrated a neutrophil leukocytosis (18·0 × 10⁹ l⁻¹) and a C-reactive protein level of 143 mg l⁻¹ (normal range <10 mg l⁻¹). Plain X-rays of the shoulder joint and a chest X-ray were normal. One set of blood cultures was taken from the patient, and he was commenced on a treatment of 1 g empirical IV flucloxacinill 6 hourly, and 1·2 g benzylpenicillin 6 hourly. His blood cultures gave a positive signal (BacT-Alert 3D BioMerieux) 24 h after admission, for a Gram-negative, oxidase-positive, beta-haemolytic bacillus identified as A. veronii biovar sobria from two bottles (API 20E; 7176755, bioMérieux). The isolates antimicrobial susceptibility was determined by the British Society for Antimicrobial Chemotherapy disc diffusion method (BSAC standardized disc susceptibility method, version 4, 2005; www.bsac.org.uk/susceptibility_testing.cfm) and resistance to ampicillin, and susceptibility to cefuroxime, cefotaxime, ciprofloxacin, gentamicin, trimethoprim and imipenem was found. Ciprofloxacin was substituted for penicillin and flucloxacinill, the patient receiving 16 days intravenous ciprofloxacin 200 mg twice daily before switching to oral treatment, completing a total of 6 weeks antibiotic therapy. A joint wash-out procedure was performed in the operating room 48 h after admission, the patient having already received three doses of ciprofloxacin. The shoulder aspirate taken in the theatre demonstrated pus cells (20–100 pus cells mm⁻³) but no organisms on Gram staining, and aerobic and anaerobic cultures remained negative after 5 days incubation. Stool cultures were also negative. Over the following 10 days, the patient’s symptoms settled, he became afebrile and the inflammatory markers resolved. He was discharged to complete a 4 week course of 500 mg oral ciprofloxacin 12 hourly.

In view of the unusual blood-culture result, the joint aspirate was sent for analysis by broad-range 16S rDNA PCR. This assay is part of the routine clinical microbiology service at Great Ormond Street Hospital and the method has been
described previously (Harris et al., 2003). Briefly, nucleic acid was extracted from a 200 μl sample using a modified version of the QIAmp DNA mini kit (Qiagen) and subjected to PCR with broad-range primers that amplify DNA from almost any bacterial species (Harris et al., 2003). Appropriate positive and negative controls were processed simultaneously. The sample generated a PCR product of the correct size, and the DNA sequence of this product was determined and compared with 16S rDNA sequences in the GenBank database using the BLAST program available at the National Center for Biotechnology Information (www.ncbi.nlm.nih.gov), identifying A. veronii as the organism.

The patient was admitted to the same hospital 2 months later with an ischaemic right hand due to axillary artery thrombosis, developed multi-organ failure secondary to a gastro-intestinal bleed and died during that admission. A post-mortem showed he had developed an acute gastric ulcer, with secondary ischaemic heart disease. There was no evidence of underlying malignancy.

**Discussion**

*Aeromonas* bacteraemia is rare and in a review of 59 cases, the largest to date, just 6 cases (17 %) were due to *A. veronii* biovar sobria (Ko et al., 1995). A previous case of septic arthritis due to *A. veronii* biovar sobria has been published (Steinfeld et al., 1998), this being the first to report on the technique of broad-range 16S rDNA PCR in the identification of this organism. The recent literature on *Aeromonas* bacteraemia, listed in Table 1, identifies some key features. Three species account for the majority of human infections with bacteraemia: *A. hydrophila* (68 %), *A. veronii* biovar sobria (17 %) and *A. caviae* (10 %) (Ko et al., 1995). The relative preponderance of *A. veronii* biovar sobria in bacteraemic patients and the high case fatality rate suggest that *A. veronii* biovar sobria is more pathogenic than the other species, a hypothesis supported by animal studies (Daily et al., 1981; Janda et al., 1985).

The clinical circumstances and microbiology strongly suggest that the patient developed a bacteraemia followed by seeding to the shoulder joint leading to septic arthritis. It is unsurprising that cultures of joint pus were negative, since the patient had received three doses of ciprofloxacin prior to joint aspiration. Detection of *A. veronii* DNA in the shoulder aspirate correlated with the earlier blood-culture result and highlights the utility of broad-range 16S rDNA PCR in the identification of a causative organism in culture negative specimens, especially following the administration of antibiotics. The use of broad-range 16S rDNA PCR in diagnostic clinical microbiology is increasingly described in the literature (Rantakokko-Jalava et al., 2000; Harris et al., 2003). The assay results can be used to determine an appropriate antibiotic therapy, usually the rationalization of broad-spectrum antibiotic treatment. This reduces potential side effects for the patient, slows the development of antibiotic resistance and reduces the development of multi-drug resistant organisms.

**Table 1. Summary of recent publications on *Aeromonas* spp. and *A. sobria* bacteraemia**

<table>
<thead>
<tr>
<th>Reference</th>
<th>Year</th>
<th>Species</th>
<th>Acquisition</th>
<th>Underlying disease</th>
<th>Age/sex*</th>
<th>Case fatality</th>
</tr>
</thead>
<tbody>
<tr>
<td>Janda &amp; Breuden (1987)</td>
<td>1987</td>
<td><em>Aeromonas</em> spp.</td>
<td>100% monomicrobial</td>
<td>100% community</td>
<td>50 M: 5 F</td>
<td>33%</td>
</tr>
<tr>
<td>Ko et al. (1995)</td>
<td>1995</td>
<td><em>Aeromonas</em> spp.</td>
<td>100% monomicrobial</td>
<td>100% community</td>
<td>50 M: 5 F</td>
<td>33%</td>
</tr>
<tr>
<td>Janda &amp; Abbott (1998)</td>
<td>1998</td>
<td><em>Aeromonas</em> spp.</td>
<td>21–31% (17%)</td>
<td>100% community</td>
<td>50 M: 5 F</td>
<td>33%</td>
</tr>
<tr>
<td>Steinfeld et al. (1998)</td>
<td>1998</td>
<td><em>Aeromonas</em> spp.</td>
<td>100% monomicrobial</td>
<td>100% community</td>
<td>50 M: 5 F</td>
<td>33%</td>
</tr>
<tr>
<td>Janda &amp; Kristiansen (2001)</td>
<td>2001</td>
<td><em>Aeromonas</em> spp.</td>
<td>100% monomicrobial</td>
<td>100% community</td>
<td>50 M: 5 F</td>
<td>33%</td>
</tr>
<tr>
<td>Llopis et al. (2004)</td>
<td>2004</td>
<td><em>Aeromonas</em> spp.</td>
<td>100% monomicrobial</td>
<td>100% community</td>
<td>50 M: 5 F</td>
<td>33%</td>
</tr>
</tbody>
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*F, Female; M, male.
resistance and makes the test cost efficient. (The assay costs around £50 per sample.) In this case, antibiotic treatment had been changed following the positive blood culture results, before the joint aspiration and PCR were performed. However, in general the assay would be useful for diagnosing culture-negative septic arthritis, either with or without positive blood culture results, and if used, management could be altered in a more timely fashion.

A further interesting aspect to this case, unlike other cases of Aeromonas bacteraemia, is the absence of previously recognized risk factors for infection: chronic liver disease, malignancy, chronic lymphocytic leukaemia, diabetes or immunosuppressive drugs (Ko et al., 1995). His only past medical history was axillary artery thrombosis and an excised basal cell carcinoma. He was not on medication, and on enquiry, it was reported that there was no change in his bowel habit or abdominal pain. Investigations for underlying disease were negative with normal haematological parameters, and differential white cell count, liver function, glucose, immunoglobulin profile and chest X-ray. A review by Ko et al. (1995) indicated a male preponderance (4 male:1 female) for the disease and less virulence in the elderly. No focus of infection for the bacteraemia was identified, though it has been suggested that translocation across the bowel is the most likely source (Thomsen et al., 2001). However, Ko et al. (1995) found that only 5% of patients with a positive blood culture had diarrhoea. The original source may be ingestion of contaminated water or food, especially undercooked fish. Stool samples in our case were negative and there was no history of contact with potentially contaminated water or food products.

This report identifies an unusual organism, with confirmation of septic arthritis by a novel technique, raising the possibility that such cases are underreported and may occur, contrary to previous reports, in the absence of obvious risk factors.

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References