Case Report

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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 fresh water, 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 ($18.0 \times 10^6 \mu\text{L}^{-1}$) 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 flucloxacillin 6 hourly, and 1.2 g benzylpenicillin 6 hourly. His blood cultures gave a positive signal (BacT-Alert 3D BioMérieux) 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 flucloxacillin, 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

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

Reference	No./percentage of <i>Aeromonas</i> that are <i>A. sobria</i>	Species	Acquisition	Age/sex*	Underlying disease	Case fatality
Janda & Brenden (1987)	6/13 (46 %)	100 % monomicrobial	100 % community	70/ 5 M: 1 F	2 cirrhosis; 2 haematological; malignancies; 1 neoplasm; 1 diabetes	33 %
Ko <i>et al.</i> (1995)	10/59 (17 %)	56 % polymicrobial	69 % community	–	48 % cirrhosis; 24 % all neoplasms; 10 % diabetes	56 %
Janda & Abbott (1998)	23–31 % (review)	–	–	–	40–50 % malignancy; 15–30 % cirrhosis; 3–5 % diabetes	–
Steinfeld <i>et al.</i> (1998)	1 (case report)	Monomicrobial	Community	31 M	Cirrhosis	Recovered
Thomsen & Kristiansen (2001)	3 (case series)	1 polymicrobial (<i>Escherichia coli</i>)	3/3 community	33 M; 75 M; 81 M	1 malignancy; 1 haematological; malignancy; 1 HIV	Recovered
Llopis <i>et al.</i> (2004)	7/75 (9 %), 16/75 (21 %) (genus only)	28 % polymicrobial (<i>Escherichia coli</i>)	71 % community	61/ 72 % M	36 % cirrhosis; 33 % malignancy	36 %

*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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None of the authors has any potential conflicts of interest.

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