Case Report

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A 15-year-old male patient with a severe chronic haemolytic anaemia (elliptocytosis) presented to the emergency department of our paediatric hospital (Hôpital Armand-Trousseau) with a fever of 39 °C, a painful left knee lameness and a left thumb oedema since the previous day. Before the boy was 4 years old, an elective splenectomy was first performed in January 1999, and then a total splenectomy in October 2000.

At the emergency department (Hôpital Armand-Trousseau), a painful limitation of the knee joint was observed on physical examination, and investigated by aspiration of the knee joint: one aliquot of the aspirate was injected directly into an aerobic broth bottle and analysed using the automated microbial detection system BacT/ALERT (bioMérieux), and additional aliquots were cultured on agar plates (blood agar and chocolate agar) combined with enrichments in two broth bottles (Hemoline; bioMérieux). A blood culture was also performed. The boy was hospitalized, and treated for 24 h with an intravenous antimicrobial combination of cefotaxime (60 mg kg⁻¹ per day) and rifampicin (40 mg kg⁻¹ per day), as well as analgesic drugs. Laboratory findings showed a white blood cell (WBC) count of 23 300 WBCs nl⁻¹, a C-reactive protein level of 67 mg l⁻¹ and a procalcitonin level of 1.30 ng ml⁻¹. Afterwards, a left ankle oedema appeared, as well as a generalized jaundice with haemolysis characteristics (haemoglobin=8 g dl⁻¹, reticulocytes=115 000 nl⁻¹, haptoglobin=low level, and free bilirubin=155 µg l⁻¹). A spontaneous decrease of the haemolytic anaemia was subsequently observed. During the hospital stay, on day 1, a synovial biopsy was performed after a surgical knee joint washing, showing a richly vascularized chorion with many neutrophils, and a poor inflammatory response in the aponeurotic tissue resulting in only weakly inflamed tissue. During this operation, two new BacT/ALERT broth bottles (aerobic/anaerobic) were also directly injected with the joint aspirate specimen. After 24 h, a decrease of the clinical symptoms, such as fever, knee and joint pain, and oedema, was observed, as well as a decrease of the inflammatory syndrome (WBCs=13 000 nl⁻¹, C-reactive protein level=10 mg l⁻¹). The outcome was favourable and the boy was discharged with only an analgesic treatment and the usual penicillin prophylaxis for asplenic patients (2 000 000 IU per day).

Microbiological investigation

Examination of the synovial fluid showed 10⁶ WBCs nl⁻¹ with 80 % neutrophils but no micro-organism observed by Gram-stained smear. The aerobic joint fluid BacT/ALERT bottle set up whilst the patient was in the emergency department grew in 72 h, showing small, coccoïd Gram-negative rods. Subcultures of the positive bottle grew in 48 h when inoculated on 5 % sheep blood agar plates and incubated in 5 % CO₂, showing small colonies surrounded by a zone of browning. Oxidase and catalase reactions were

Abbreviation: WBC, white blood cell.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of B. holmesii is JN127352.
negative. Routine identification procedures [32GN strip (bioMérieux) and Gram-negative Vitek card (bioMérieux)] were unsuccessful and, respectively, misidentified the organism as Moraxella lacunata with 97.6% probability (good identification confidence level) and as Aeromonas salmonicida/Oligella ureolytica (low discrimination). The isolate was subsequently identified as Bordetella holmesii by 16S RNA gene sequence analysis as described by Négre et al. (2004). The 16S RNA gene sequence of B. holmesii isolate has been submitted to the GenBank nucleotide database under GenBank accession number JN127352. This sequence (452 bp) showed maximum similarity, 100% (452/452 nt) with the 16S rRNA gene from the B. holmesii reference strain CDC F5101 (GenBank accession no. NR_029173), as well as eight other B. holmesii isolates, and 99% (449/452 nt) with the reference strains, Bordetella bronchiseptica RB50 (GenBank accession no. BX640449), Bordetella pertussis strain Tohama I (GenBank accession no. BX640418) and B. pertussis CS (GenBank accession number no. CP002695). The complete B. holmesii identification was achieved at the Centre National de Référence (CNR) de la Coqueluche et Autres Bordetelloises, Institut Pasteur. The CNR confirmed the identification by performing B. holmesii specific recA PCR according to Antila et al. (2006). The bacterium does not express any of the virulence determinants such as pertussis toxin, adenylate cyclase-haemolysin, filamentous haemagglutinin, pertactin or fimbriae using immunoblot and specific adenylate cyclase-haemolysin, filamentous haemagglutinin, as well as eight other Bordetella pertussis factors. MICs determined by Etest on Mueller–Hinton agar with 0.5 mg l⁻¹ for amoxicillin, 0.19 mg l⁻¹ for ticarcillin–clavulanate, 0.016 mg l⁻¹ for piperacillin–tazobactam, 32 mg l⁻¹ for cefotaxime and ceftriaxone, 0.094 mg l⁻¹ for ceftazidime and ciprofloxacin, 0.75 mg l⁻¹ for imipenem, 1 mg l⁻¹ for tobramycin and 4 mg l⁻¹ for amikacin. Testing for β-lactamase with the nitrocefin test was negative. The isolates are susceptible to rifampicin. Standard protocols, including Hemolone bottles, were culture negative, as well as BacT/ALERT bottles inoculated with the patient’s joint fluid set up whilst the patient was in the operating room (Hôpital Armand-Trousseau). Of interest, the real-time PCR detection of B. pertussis/B. holmesii species based on IS481 detection performed retrospectively on DNA extract from the joint fluid as described elsewhere (Templeton et al., 2003; Bidet et al., 2008) was positive, confirming the presence of the pathogen in the knee joint. The blood culture was negative.

**Discussion**

B. holmesii is a small Gram-negative rod belonging to the genus Bordetella, which currently includes nine species [B. pertussis (causative agent of whooping cough), Bordetella parapertussis, Bordetella avium, Bordetella trematum, B. bronchiseptica, Bordetella hinzii, Bordetella petrii and ‘Bordetella ansporii’] (Ko et al., 2005). The taxonomic name ‘B. ansporii’ was not validly published at the time of submission (Ko et al., 2005) and at the time of writing still does not appear in the List of Prokaryotic Names with Standing in Nomenclature (http://www.bacterio.cict.fr/). B. holmesii causes strictly human infections, with no known reservoir. The role of B. holmesii in respiratory tract colonization or infection remains to be elucidated. Modes of acquisition also remain to be identified. B. holmesii was first described in 1995 as a cause of septicaemia in 15 patients (Weyant et al., 1995). Subsequently, B. holmesii has been reported as a cause of bacteraemia, endocarditis and pneumonia, mainly in compromised and, more particularly, in asplenic patients with often anatomical or functional asplenia, such as patients with sickle cell disease (Lindquist et al., 1995; Morris & Myers, 1998; Tang et al., 1998; Njamkepo et al., 2000; Greig et al., 2001; Russell et al., 2001; Shepard et al., 2004; Dörbecker et al., 2007; McCavit et al., 2008; Panagopoulos et al., 2010). The clinical course usually remained uneventful, and patients tended to recover without complications. In our case, the clinical course was also uneventful. It has been predicted that there is a seasonal (autumn–winter) predominance for the illness-onset associated with B. holmesii infections (Shepard et al., 2004). In most reports, species identification by commercial systems failed, and, more recently, a paper reported a systematic misidentification of B. holmesii as Acinetobacter lwoffii using the Vitek2 system (Panagopoulos et al., 2010). The ID 32GN test strip did not allow a reliable identification of B. holmesii since the database of this commercially available diagnostic kit only includes B. bronchiseptica, and does not cover the other species of the genus Bordetella. The Gram-negative Vitek card only identifies B. bronchiseptica and B. trematum in the genus Bordetella. The most accurate identification tool seemed to be 16S rRNA gene sequence analysis. However, the use of alternative genes to 16S rRNA gene has been reported for identification of Bordetella, namely the outer-membrane protein A gene (ompA) and putative response regulator protein (risA) genes, originally by Von Wintzingerode et al. (2002) and also by Fry et al. (2005, 2007).

In our case, the positivity of the IS481 real-time PCR indicates the usefulness of this method to search for B. holmesii, a species that like B. pertussis harbours IS481 (Templeton et al., 2003). However, molecular techniques like 16S rRNA gene-based PCR could be useful, as in many cases, the culture result may be falsely negative for various reasons, the most frequent being patients starting antibiotic treatment before adequate samples are taken. In most of the case reports, the MICs for high β-lactams observed for B. holmesii strains, including third-generation cephalosporins, suggest that these drugs are not optimal for the treatment of B. holmesii infection (Shepard et al., 2004). MICs of erythromycin were higher than those obtained for susceptible B. pertussis isolates, suggesting that erythromycin may be less active against B. holmesii (Hill et al., 2000). In contrast, low MICs of carbapenems and fluoroquinolones indicate the potential usefulness of these drugs for treatment. However, additional data are needed to elucidate the meaning of in vitro susceptibility data for this organism and for deciding when antimicrobial therapy...
is necessary. In our case report, the patient was treated for 24 h with cefotaxime, which seemed ineffective in vitro, but the patient promptly recovered perhaps due to the concomitant administration of rifampicin and a spontaneously favourable evolution. Determinants of *B. holmesii* virulence are incompletely elucidated. *B. holmesii* is endowed with a factor highly related to filamentous haemagglutinin, a prominent virulence factor of the well-characterized pathogenic bordetellae (Link et al., 2007). Whether *B. holmesii* strains possess a capsule or not is still unknown (Panagopoulos et al., 2010). Regarding host factors, the increased susceptibility to infection and overwhelming sepsis, predominantly with encapsulated bacteria, is well known in asplenic patients.

In conclusion, if asplenia may constitute a risk factor for *B. holmesii* infection, much remains to be learned about its mode of transmission, pathogenesis and natural history. Clinicians, especially when treating patients with asplenia, should be aware of the capacity of this organism to cause disease and be alert to potentially new clinical manifestations of infection with this micro-organism. To our knowledge, this is the first report of *B. holmesii* septic arthritis in an asplenic patient.

References


