Case Report

Prosthetic valve endocarditis caused by *Bordetella holmesii*, an *Acinetobacter* lookalike

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We report a case of fulminant endocarditis on a prosthetic homograft aortic valve caused by *Bordetella holmesii*, which was successfully managed by surgical valve replacement and antibiotic treatment. *B. holmesii*, a strictly aerobic, small, Gram-negative coccobacillus, has been implicated as an infrequent cause of a pertussis-like syndrome and other respiratory illnesses. However, *B. holmesii* is also a rare cause of septicemia and infective endocarditis, mostly in immunocompromised patients. To our knowledge, this is the first report of *B. holmesii* endocarditis on a prosthetic aortic valve. Routine laboratory testing initially misidentified the strain as *Acinetobacter* sp. Correct identification was achieved by 16S rRNA gene and outer-membrane protein A (*ompA*) gene sequencing. Interestingly, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry also produced an accurate species-level identification. Subsequent susceptibility testing and review of the literature revealed ceftazidime, cefepime, carbapenems, aminoglycosides, fluoroquinolones, piperacillin/tazobactam, tigecycline and colistin as possible candidates to treat infections caused by *B. holmesii*.

**Case report**

A 36-year-old man, known as a heavy smoker and an excessive alcohol consumer, was admitted to the emergency department with a septicaemia-like clinical presentation, dyspnoea and oedema of the lower limbs. On arrival, he suffered from an acute lung oedema due to septic shock. He complained of general malaise for the previous 2 weeks and of a chilling fever for the previous 3 days. His white blood cell count was 14.5 x 10^9 l^-1 (reference range 3.7–9.5 x 10^9 l^-1 ) with 89% neutrophils (reference range 46–64%), blood haemoglobin level 97 g l^-1 (reference range 133–167 g l^-1) and platelet count 306 x 10^9 l^-1 (reference range 150–450 x 10^9 l^-1). He had a serum creatinine level of 6.4 mg l^-1 (reference range 0.67–1.17 mg l^-1) and a C-reactive protein level of 124 mg l^-1 (reference range <5 mg l^-1). D-dimers were elevated at 2740 µg l^-1 (reference range <300 µg l^-1). The cardiac markers troponin T and the MB isoenzyme of creatinine kinase were both increased to 0.11 µg l^-1 (reference range <0.03 µg l^-1) and 11.3 µg l^-1 (reference range <6.7 µg l^-1), respectively. He had a medical history of infectious endocarditis on a post-rheumatic aortic and mitral valve caused by *Streptococcus bovis* 7 years before. This had been managed by antibiotic treatment, replacement of the aortic valve by a homograft, and replacement of the mitral valve by a mechanical valve using a Bentall procedure.

Transoesophageal echocardiography revealed leaflet vegetations of 1 cm on the aortic homograft with massive aortic regurgitation due to the destruction of one leaflet. There were no signs of prosthetic valve endocarditis of the mitral valve. Empirical antibiotic treatment consisting of vancomycin, gentamicin and fluoroxacillin was initiated. Three days after admission, the damaged aortic homograft was replaced by a mechanical composite graft (St Jude Regent, 23 mm) during a redo Bentall procedure. The patient was successfully treated.

**Abbreviation**: MALDI-TOF, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry.
developed a septicaemia-based acute renal failure, with creatinine levels peaking at 55 mg l⁻¹ at day 5 after admission. Before antibiotics were initiated, four aerobic and four anaerobic blood culture bottles (Bact/Alert System; bioMérieux) were collected and after a mean incubation time of 78 h (range 76.7–78.8 h) at 37 °C, thin, rod-shaped, Gram-negative bacteria were observed in three aerobic blood cultures. Based on these findings, antibiotic therapy was switched to meropenem with doses adjusted to renal function. Further bacteriological investigation showed non-motile, slow-growing, strictly aerobic, asaccharolytic, oxidase-negative, catalase-positive coccobacilli. The isolate grew on blood and chocolate agar after 2 days and after about 4 days on MacConkey agar plates in ambient air. The strain was tested using the API 20 NE gallery (bioMérieux), which produced a presumptive identification of Acinetobacter sp. with an 80 % probability of correct identification. Susceptibility testing of the organism was performed using Etest (bioMérieux) on Mueller–Hinton II agar (Becton Dickinson). A soluble brown pigment was detected on these plates after 2 days of incubation at 37 °C. Because of slow growth, MICs were determined after 48 h, as recommended by the manufacturer, and the median value of different observers (n=11) was used (see Table 1).

Molecular identification was obtained by extracting bacterial DNA from colonies grown on blood agar (homemade Columbia agar supplemented with 5 % horse blood) using the QIAamp DNA Mini kit (Qiagen) in accordance with the manufacturer’s instructions. The bacterial 16S rRNA gene was amplified using the following primers: forward 15.1 (3′-AGA GTT TGA TCC TGG CTC AG-3′; Escherichia coli position 8–27) and reverse 15.2 (5′-CTT GTG CGG GTC CCC GTC AAT TC-3′; E. coli position 938–926) (Baker et al., 2003). Amplification products of approximately 930 bp were checked by agarose gel electrophoresis (2 %) visualized by ethidium bromide staining. Sequencing of the amplicon was done using the ABI Big Dye Terminator sequencing kit (Applied Biosystems) and analysed on the ABI 3130XL 16-capillary electrophoresis apparatus (Applied Biosystems). Assembly of the different fragments was carried out by using the Chromas Pro software (Technelysium, Tewantin, QLD), whereafter the obtained sequence was compared to all known sequences in GenBank by using the Basic Local Alignment Search Tool (BLAST) at the website of the National Center for Biotechnology Information (NCBI) (http://blast.ncbi.nlm.nih.gov). The BLAST search for the query sequence of 823 nt resulted in a 100 % similarity with eight different entries in GenBank of Bordetella holmesii. The species with the next highest similarity, 99 %, was Bordetella pertussis (819/823 nt). Identification was confirmed by outer-membrane protein A (ompA) gene sequencing conducted at the National Reference Centre for Bordetella pertussis. PCR amplification and sequencing was performed as previously described with the primers omp1e and omp2b (von Wintzingerode et al., 2001; Fry et al., 2007). PCR products were purified and sequenced by the VIB Genetic Service Facility (University of Antwerp, Wilrijk, Belgium) using an ABI 3730 DNA sequencer (Applied Biosystems). Sequence assembly was performed by using the BioNumerics sequence type module (BioNumerics v6.5; Applied Maths). The consensus sequence was compared with the GenBank database by BLASTN interrogation (http://blast.ncbi.nlm.nih.gov). The 680 nt of the ompA sequence from strain M10-4183 showed 100 % similarity with the ompA sequence from the type strain (AM748266, 490/490 nt) and a non-type strain (AM748267, 558/558 nt) from B. holmesii. The species with the next highest similarity, 93 %, was Bordetella hinzi (AM748265, 584/625 nt). Also, identification was verified by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS; Microflex, Bruker Daltonik) by the direct transfer method and in combination with an in-house database (log score best match B. holmesii 2.176; second best match Bordetella petrii log score 1.485) (A. De Bel, unpublished data). Biochemical identification tests, including cellular fatty acid analysis, were in agreement with the characteristics of the genus Bordetella and the species B. holmesii (Weyant et al., 1995; Wirsing von König et al., 2011).

 Clinically, the patient made a remarkable recovery with a gradual recuperation of kidney function without requiring dialysis. After 19 days of hospitalization, the patient was discharged in good health.

**Table 1. In vitro susceptibility of the Bordetella holmesii isolate as determined by Etest on Mueller–Hinton II agar**

<table>
<thead>
<tr>
<th>Antimicrobial agent</th>
<th>MIC (mg l⁻¹)</th>
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<tr>
<td>Penicillin</td>
<td>&gt;32</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>0.25</td>
</tr>
<tr>
<td>Cefuroxime</td>
<td>&gt;256</td>
</tr>
<tr>
<td>Cefotaxime</td>
<td>&gt;32</td>
</tr>
<tr>
<td>Ceftazidime</td>
<td>≤0.5</td>
</tr>
<tr>
<td>Cefepime</td>
<td>≤0.25</td>
</tr>
<tr>
<td>Meropenem</td>
<td>0.032</td>
</tr>
<tr>
<td>Piperacillin/tazobactam</td>
<td>≤0.016</td>
</tr>
<tr>
<td>Temocillin</td>
<td>12</td>
</tr>
<tr>
<td>Moxifloxacin</td>
<td>0.032</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>0.50</td>
</tr>
<tr>
<td>Tigecycline</td>
<td>0.094</td>
</tr>
<tr>
<td>Colistin</td>
<td>≤0.016</td>
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</tbody>
</table>

**Discussion**

Here we report a case of endocarditis of an aortic homograft caused by B. holmesii. This former ‘CDC nonoxidizer group 2’ (NO-2) bacterium was first isolated in 1983 and received its current name in 1995 in honour of Barry Holmes for his contributions to bacteriology (Weyant et al., 1995). Since 1995, several articles have reported the potential clinical manifestations of B. holmesii infections, including bacteraemia, endocarditis and respiratory infections. Although severe infection can occur
(Russell et al., 2001), cases of B. holmesii infection generally present with relatively mild symptoms and an uncomplicated outcome. The patient in this case, however, was admitted to the intensive care unit due to prominent heart failure and septicaemia, but after surgical and antibiotic treatment, a quick and favourable recovery followed.

The patients in many published cases of B. holmesii bacteraemia were functionally or anatomically asplenic at the time of infection. If not, another underlying immunocompromising condition like steroid therapy, diabetes, Hodgkin's disease or AIDS was frequently observed (Shepard et al., 2004). In our patient, none of these risk factors were present, although chronic and excessive alcohol abuse together with the presence of an aortic prosthetic valve may have been contributing factors. Nevertheless, this case demonstrates that infection with B. holmesii can cause a potentially life-threatening infection, even in patients without previously identified risk factors. It should be noted that, as in about three-quarters of published cases, B. holmesii affected a young adult during winter time.

Laboratory identification of the organism is challenging and we were initially unable to properly identify the isolate through routine laboratory protocols or using the API 20 NE gallery. B. holmesii typically produces a brown, soluble pigment (Weyant et al., 1995), and the only other organism known to do this is Moraxella canis (Jannes et al., 1993; Vaneechoutte et al., 2000). However, this brown pigment was only noticed on the Mueller–Hinton II plates used for susceptibility testing, especially on the border of the inhibition zone. The use of a Gram-negative identification card on a Phoenix automated system (Becton Dickinson) yielded no identification due to insufficient growth in the control well. Based on the available test results, a presumptive identification of Acinetobacter sp. was made, although delayed growth on MacConkey agar did not completely match this result. A correct identification was finally obtained by 16S rRNA gene sequence analysis. However, discrimination between B. holmesii and other Bordetella species, including B. pertussis and Bordetella bronchiseptica, is based on a limited variation in the 16S rRNA gene sequence. Other targets, such as the Bordetella ompA gene, which has a greater mutational variation than the 16S rRNA gene, may generate a more unambiguous discrimination between Bordetella species (von Wintzingerode et al., 2001; Fry et al., 2007). Indeed, ompA gene sequencing at the National Reference Centre for Bordetella pertussis confirmed the identification of B. holmesii with a much better discrimination from other Bordetella species. Interestingly, MALDI-TOF MS, a much faster and less time-consuming technique, also produced a reliable identification. This troublesome identification underlines the diagnostic value of sequencing-based techniques (Bosshard et al., 2006) and newer and fast techniques such as MALDI-TOF MS. Also, it raises the question of whether some of the assumed Acinetobacter endocarditis cases previously described were in fact caused by B. holmesii, particularly in times when molecular identification was not yet available.

There is no consensus regarding the treatment of B. holmesii infection. Furthermore, since infection with B. holmesii is generally not severe, the indications for antibiotic treatment are unclear. Various techniques have been used to determine the in vitro susceptibility of B. holmesii, including broth microdilution (Shepard et al., 2004), Etest (Panagopoulos et al., 2010), automated systems (Lam et al., 2008), agar dilution (Tang et al., 1998) and disc diffusion (Njamkepo et al., 2000). A variable susceptibility to β-lactams has been reported. Moreover, controversy exists about susceptibility to cefotaxime, a third-generation cephalosporin, which has been reported both active (Tang et al., 1998; Njamkepo et al., 2000) and not active (Shepard et al., 2004; Panagopoulos et al., 2010; Lam et al., 2008). We performed susceptibility testing by Etest and found a high MIC of cefotaxime, confirming the results of Shepard et al. (2004), who tested the largest cohort of clinically important isolates so far. For ceftazidime, however, we found a low MIC. This is in agreement with its previously reported in vitro susceptibility, indicating that this third-generation cephalosporin may have clinical utility (Panagopoulos et al., 2010; Lam et al., 2008; Tang et al., 1998). Nevertheless, further research is needed to determine the most effective antimicrobial treatment and for the determination of breakpoints to be used for in vitro susceptibility testing. Such studies are, however, difficult to perform as severe infections due to B. holmesii are rare.

We can conclude that endocarditis caused by B. holmesii is – as far as we know – a very rare condition and, to our knowledge, this is the first report of prosthetic valve endocarditis due to this organism. Nevertheless, clinical laboratories should take B. holmesii into consideration when blood cultures produce slowly growing, aerobic, asaccharolytic, catalase-positive, oxidase-negative, Gram-negative bacilli obtained from a patient suffering from suspected or proven endocarditis, even when no apparent immunosuppression is present. Also, clinicians should be made aware of its variable susceptibility to β-lactams.

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References


