A UK clinical isolate of *Bordetella hinzii* from a patient with myelodysplastic syndrome

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What is believed to be the first clinical isolate of *Bordetella hinzii* in the UK, from a patient with myelodysplastic syndrome, is described. This patient had no known avian exposure, and the source of the organism remains unknown. It appears that the underlying immune deficiency of the patient increased the susceptibility to opportunistic infection with this organism. Human infection with *B. hinzii* is rare and this species is difficult to differentiate from *Bordetella avium* by routine phenotypic methods. Confirmation can be reliably achieved using genotypic methods, and the greater mutational variation of the ompA gene compared to other genes (e.g. 16S rRNA gene) allows unambiguous identification of this and other non-classical *Bordetella* species.

Case report

A 79-year-old male with myelodysplastic syndrome was admitted to hospital for a blood transfusion. On admission blood tests revealed the following: 6.2 g haemoglobin dl⁻¹, 0.4 × 10⁹ white blood cells l⁻¹ (0.1 × 10⁹ neutrophils l⁻¹, 0.3 × 10⁹ lymphocytes l⁻¹, and monocytes, eosinophils and basophils all at 0.0 × 10⁹ l⁻¹). He had experienced malaise and flu-like symptoms for 1 week, and had been commenced on oral co-amoxiclav 500/125 (500 mg amoxicillin as trihydrate, 125 mg clavulanic acid as potassium salt) three times daily by his general practitioner 2 days earlier for a presumed chest infection. On admission his symptoms had improved and the clinical examination was unremarkable. The co-amoxiclav was discontinued. His previous medical history included diabetes mellitus, hypertension and Paget’s disease. On the second day of admission he developed a fever of 38.4 °C prior to a blood transfusion. On examination he had a pulse rate of 71 beats min⁻¹, blood pressure of 141/55 mmHg, a respiratory rate of 18 breaths min⁻¹ and O₂ saturation of 95 % on air. Blood was taken for bacteriological culture before commencing therapy with intravenous vancomycin 1 g twice daily and intravenous ceftazidime 2 g twice daily. The following day his C-reactive protein had risen to 159 mg l⁻¹ from 70 mg l⁻¹ on admission. The patient was breathless but his chest X-ray was normal. The patient had no central venous catheters in situ and responded well to the empirical antibiotic therapy. Vancomycin was discontinued after 5 days and ceftazidime after 7 days. The patient recovered and was discharged home 13 days after admission. The patient had no known avian exposure.

Methods

**Microbiological investigation.** A blood sample for culture was taken via venepuncture of a peripheral vein and inoculated into a standard set of one aerobic and one anaerobic Bact/ALERT bottle (bioMérieux),
and incubated at 37 °C in a BacT/ALERT 3D automated blood culture system (bioMérieux). Bottles signalling positive were sampled aseptically and inoculated as described previously (Fry et al., 2007). A film was made for Gram-staining and antimicrobial susceptibility testing was performed using the disc diffusion method according to the British Society for Antimicrobial Chemotherapy (BSAC) guidelines (Andrews, 2006). The aerobic bottle signalled positive after 49 h and the anaerobic bottle remained negative after 5 days incubation.

A further 24 h incubation on solid media revealed a pure growth of a strictly aerobic Gram-negative bacillus. Results of initial testing of the isolate by the submitting laboratory revealed the following phenotypic characteristics: positive reaction for oxidase and motility using the hanging drop method at 37 °C, and a negative reaction for urease production. The API 20NE commercial identification kit (bioMérieux) gave an analytical profile number of 0000067, and a good identification for Bordetella avium with a 96.6 % confidence interval, and the isolate was referred to the Respiratory and Systemic Infection Laboratory (RSIL) for confirmation (designated strain H062540035).

Phenotype analysis. The following characteristics were examined by the RSIL: colonial appearance, growth on charcoal blood agar and MacConkey agar, oxidase and slide agglutination with Bordetella pertussis and Bordetella parapertussis polyvalent antisera (Difco).

Genotype analysis. Genomic DNA extraction, PCR amplification, sequencing and preliminary analysis of the gene encoding the Bordetella outer-membrane protein A (ompA) and small-subunit (SSU) rRNA were as described in published work (von Wintzingerode et al., 2002; Fry et al., 2005). Contiguous sequence assembly and sequence analyses were performed using BioNumerics, version 4.5 beta 7 (Applied Maths). The consensus sequence was compared with public databases using the BLASTN program (http://www.ncbi.nlm.nih.gov/BLAST/) (McGinnis & Madden, 2004) and sequences with the greatest similarity downloaded for further analysis. In addition to the sequences determined in this study, as described below, sequences with the following accession numbers were also used: AM167904, BX640413, BX640447, AI242599, AJ920264, EF368182, AM275334, AY594191, AF177667, BX640432, AI277798, AI278450, BX640449, BX640420, DQ091361, AF366579, AY466114, U04820, AI249861, EF212440, AJ870969 and AY594190. Phylogenetic trees were constructed using MrBayes version 3 (Ronquist & Huelsenbeck, 2003). The ompA tree was created from 100 000 generations (25 % discarded as burn-in) of three heated and one cold chain with 6 substitution models distributed rate variation and a proportion of invariant sites, and converged with a mean SD of split frequencies of 0.011. The decision to exclude the proportion of invariant sites option from the gamma distributed rate variation of the ompA analysis was taken following assessment of the ompA dataset with MODELTEST (Posada & Crandall, 1998). In addition to the ompA and SSU rDNA gene of strain H062540035, the ompA sequences of five reference strains, including the type strain (NCTC13199) and a non-type strain of B. hinzii (NCTC13200), the type strain (NCTC12912) and a non-type strain of Bordetella holmesii (NCTC13202), and the type strain of Bordetella trematud (NCTC12995) were also determined. These sequences have been submitted to the GenBank/EMBL/DDJB nucleotide sequence databases under accession numbers AM748263–AM748269.

Results
Preliminary test results at the RSIL on strain H062540035 were consistent with members of the genus Bordetella. The organism could be cultured on both MacConkey agar and charcoal blood agar, and colonies had the following phenotypic characteristics: positive reaction for oxidase and negative for slide agglutination with B. pertussis and B. parapertussis antiserum (Difco). Results of antimicrobial susceptibility testing by the hospital laboratory were: susceptible to amoxicillin, co-amoxiclav, co-trimoxazole, gentamicin, ceftazidime and meropenem, and resistant to cefuroxime, ceftriaxone and ciprofloxacin.

The ompA sequence from strain H062540035, showed maximum similarity, 100 % (600/600 nt), with the ompA gene from the type strain of B. hinzii NCTC13199 (GenBank/EMBL/DDJB accession no. AM748264) and 99.8 % (599/600 nt) with a non-type reference strain of the same species, NCTC13200 (accession number AM748265). The single nucleotide difference was a synonymous mutation (codon GAC to GAG, both coding for aspartic acid) in the predicted sequence of the OmpA protein. The species with the next highest similarity belonged to B. holmesii NCTC13202 (accession number AM748267), 92.9 % (455/490 nt). The SSU rRNA gene sequence from strain H062540035 (accession number AM748269), showed maximum similarity, 100 % (1411/1411 nt), with the same gene from the type strain of B. hinzii NCTC13200 (accession number AF177667). The species with the next highest similarity, 99.4 % (1402/1410 nt), belonged to B. parapertussis DSM 4922 (accession number AJ278450). The phylogenetic relationships between strain H062540035 and the Bordetella species inferred from ompA and the SSU rRNA genes are shown in Fig. 1.

Discussion
Currently, commercial systems, such as the biochemical identification API20NE strips, only include two Bordetella species, Bordetella bronchiseptica and B. avium, in their identification library. B. hinzii is rarely isolated from humans and this species is difficult to differentiate from B. avium by routine phenotypic methods. Confirmation can be reliably achieved using genotypic methods, and the greater nucleotide variation of the ompA gene compared to, for example, the 16S rRNA gene, allows unambiguous identification of this and other non-classical Bordetella species. Sequence similarity and phylogenetic analyses confirmed the identity of strain H062540035 as B. hinzii. The trees generated were similar to those already described for the genus (Ko et al., 2005; Gerlach et al., 2001).

The genus Bordetella comprises eight validly named species (von Wintzingerode et al., 2001), and one that awaits formal description, ‘Bordetella anisorpia’ sp. nov. (Ko et al., 2005; Fry et al., 2007). As in some of the previous cases, there was no known avian exposure in the case presented here, suggesting that the organism was obtained from other sources as yet unidentified. Similarly, it appears that this species is an opportunistic pathogen, and that underlying patient disease and/or an impaired immune system makes
patients susceptible to infection with these rarer non-classical *Bordetella* species. The authors would therefore recommend that the identity of all clinical isolates belonging to the non-classical *Bordetella* species, including those presumptively identified as *B. avium*, are confirmed by a reference laboratory.

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**References**


