Case Report

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.

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Introduction

Bordetella hinzii is a commensal of the respiratory tracts of fowl, and has been previously shown to have some pathogenic potential in immunocompromised humans (Cookson *et al.*, 1994; Vandamme *et al.*, 1995; Funke *et al.*, 1996; Gadea *et al.*, 2000). Isolation of *B. hinzii* from human sources remains extremely rare. Only seven instances of such isolation have been reported from six cases; two patients with AIDS (one with and one without respiratory infection) (Cookson *et al.*, 1994; Gadea *et al.*, 2000), one patient with cystic fibrosis (with sequential isolation) Funke *et al.* (1996), and one patient with fatal septicaemia (Kattar *et al.*, 2000). There are little or no details from the remaining two cases; in one, isolation was from sputum, and in the other, from an endotracheal aspirate from a farmer with no apparent disease (Vandamme *et al.*, 1995, 1996; Kattar *et al.*, 2000). We report the case of a patient who presented with myelodysplastic syndrome.

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, 0.4×10^9 white blood cells l⁻¹ (0.1×10^9 neutrophils l⁻¹, 0.3×10^9 lymphocytes l⁻¹, and monocytes, eosinophils and

basophils all at 0.0×10^9 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),

Abbreviation: RSIL, Respiratory and Systemic Infection Laboratory.

The GenBank/EMBL/DBJ accession numbers for the *Bordetella* sequences reported in this paper are AM748263–AM748269.

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 antiserum (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, AJ242599, AJ920264, EF368182, AM275334, AY594191, AF177667, BX640432, AJ277798, AJ278450, BX640449, BX640420, DQ409136, AF366579, AY466114, U04820, AJ249861, 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 types and gamma distributed rate variation, and converged with a mean SD of split frequencies of 0.010. The SSU rDNA tree required 400 000 generations (25 % burn-in) for convergence, with the same number of chains and of substitution types, but with gamma 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^T) and a non-type strain of *B. hinzii* (NCTC13200), the type strain (NCTC12912^T) and a non-type strain of *Bordetella holmesii* (NCTC13202), and the type strain of *Bordetella trematum* (NCTC12995^T) were also determined. These sequences have been submitted to the GenBank/EMBL/DDBJ 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^T (GenBank/EMBL/DDBJ 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* LMG 13501^T (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 ansorpii*' 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

Fig. 1. Phylogenetic analysis of the *Bordetella*, inferred from the *ompA* and small subunit rRNA-encoding gene showing location of the clinical isolate H062540035. The internal nodes of the trees are labelled with the posterior probability of that clade. Bar, mean nucleotide substitutions per site.

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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