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Case Report

The first UK isolate of '*Bordetella ansorpii*' from an immunocompromised patient

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What is believed to be the first clinical isolate of '*Bordetella ansorpii*' in the UK from an immunocompromised patient is described. The only previously documented isolation of '*B. ansorpii*' was following the initial culture of a single strain from an epidermal cyst of a patient in Korea.

Introduction

The genus *Bordetella* comprises eight named species, *Bordetella pertussis*, *Bordetella parapertussis*, *Bordetella bronchiseptica*, *Bordetella avium*, *Bordetella hinzii*, *Bordetella holmesii*, *Bordetella trematum*, *Bordetella petrii* (von Wintzingerode *et al.*, 2001), all of which, with the exception of *B. avium*, have been isolated from man, albeit rarely in some cases (Fry *et al.*, 2005; Parton, 2005). In 2005, a novel species of *Bordetella*, '*Bordetella ansorpii*' sp. nov., was proposed following the isolation of a single strain from the purulent exudate of an epidermal cyst from a patient in Korea (Ko *et al.*, 2005). Here we describe a second isolate of this bacterium, which is believed to be the first report of its occurrence in the UK.

Case report

An 88-year-old male, diagnosed 4 years earlier with chronic monomyelocytic leukaemia, presented to a routine haematology outpatient clinic feeling unwell. The patient was undergoing treatment for chronic monomyelocytic leukaemia, which had transformed to acute myeloid leukaemia approximately 6 months prior to this presentation. He had a Hickman line *in situ*, which had been inserted 6 months earlier and through which he had previously been receiving chemotherapy, that was due for removal. Two sets of blood samples were taken for microbiological culture. He was admitted to hospital the next day following the isolation of Gram-negative bacilli from these blood samples. On admission the patient complained of tiredness and shortness of breath on exertion, but no fever, chills or rigors. On examination he was apyrexial and there were no other significant findings. Blood tests revealed the following: 9.4 g haemoglobin dl^{-1}, 36.5×10^9 white blood cells l^{-1} (11.1×10^9 neutrophils l^{-1}; 3.6×10^9 lymphocytes l^{-1}; 21.4×10^9 monocytes l^{-1}; 0.1×10^9 eosinophils l^{-1}; 0.2×10^9 basophils l^{-1}) 14.6 mmol urea l^{-1}, 162 μmol creatinine l^{-1}, 56 mg C-reactive protein l^{-1}. Further blood cultures were taken from each of the red and white lumens of the Hickman line, prior to removal later that day. He was afebrile and felt well from the time of removal of the line (the day after the first blood culture was taken). A 7 day course of antibiotic treatment with ceftazidime and gentamicin was prescribed to which he responded well, his inflammatory markers returned to normal, and he was discharged home. Additional blood samples for culture were taken 17 days after discharge, but yielded no bacterial growth. The patient died 9 weeks later from underlying disease.

Abbreviation: % ID, percent identification.

The GenBank/EMBL/DDBJ accession nos for the *ompA*, *risA* and 16S rRNA gene sequences of strain H050680373 are AM275334, AM275335 and AM275336, respectively.

Methods

Microbiological investigation. All blood samples taken for culture were inoculated into standard aerobic and anaerobic BacT/Alert bottles (bioMérieux) and incubated at 37 °C in a BacT/Alert Classic 240 blood culture analyser (bioMérieux) for automatic monitoring. Bottles that signalled positive were sampled aseptically, and inoculated onto cysteine lactose electrolyte deficient agar (E&O Laboratories) incubated in air at 37 °C, Columbia blood agar and chocolate agar (E&O Laboratories) incubated at 37 °C in air with 5 % CO_2, and onto fastidious anaerobe agar (E&O Laboratories) incubated at 37 °C under anaerobic conditions. A film was also made for Gram-staining. Antibiotic susceptibility tests were performed using the British Society for Antimicrobial Chemotherapy disc diffusion method (Andrews, 2001) on IsoSensitest agar (E&O Laboratories) using antibiotic discs (Oxoid).

The aerobic bottle of one set of blood cultures taken in clinic yielded a mixture of three types of Gram-negative organisms after 24 h incubation. Using API20NE identification strips (bioMérieux) these

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were identified as *Stenotrophomonas maltophilia* [very good identification, percent identification (% ID) 99.9] and *Ochrobactrum anthropi* (good identification % ID 98.7). A third organism, which demonstrated slow oxidase positivity, failed to yield an acceptable profile for identification and was referred to the Health Protection Agency Centre for Infections for identification (designated strain H050680373). A second set of blood cultures taken the same day yielded *Acinetobacter* sp. (acceptable identification to genus level % ID 63.4) from the aerobic bottle. From the two further sets of blood cultures taken from the Hickman line on admission the following day, the aerobic bottle taken from the red lumen grew *O. anthropi* (API20NE 1243755 – excellent identification % ID 99.9) with an identical antibiogram as the *Ochrobactrum* isolated previously. The blood culture taken from the white lumen grew *Stenotrophomonas maltophilia* (API20NE 1432341 – excellent identification % ID 99.9) from the aerobic bottle with a similar, but slightly different antibiogram from the earlier isolate, and *Staphylococcus epidermidis* from the anaerobic bottle.

The following characteristics of strain H050680373 were also examined: colonial appearance, oxidase, motility using the hanging drop method at 37 °C, slide agglutination with *B. pertussis* and *B. parapertussis* antiserum (Difco).

Genotypical analysis. Genomic DNA extraction, PCR amplification and sequencing, and analyses of small-subunit (SSU) rRNA, *Bordetella* outer-membrane protein A (*ompA*) and the RisA response regulator-encoding (*risA*) genes were as described previously (Fry *et al.*, 2005). The nucleotide sequence of the 16S rRNA, *risA* and *ompA* genes of strain H050680373 have been submitted to the EMBL nucleotide sequence database under accession nos. AM275336, AM275335 and AM275334, respectively.

Results

Preliminary test results on the Gram-negative bacillus (strain H050680373) were consistent with those described for members of the genus *Bordetella*. Colonies had the following phenotypic characteristics: positive reaction for oxidase and motility using the hanging drop method at 37 °C, and negative for slide agglutination with *B. pertussis* and *B. parapertussis* antiserum. The results of antibiotic susceptibility testing for this organism were: resistant to aztreonam, ceftriaxone and cefuroxime, and sensitive to amoxicillin, augmentin, ceftazidime, chloramphenicol, ciprofloxacin, gentamicin, imipenem, tazocin and trimethoprim.

A partial region of the SSU rRNA sequence was determined, 539 nt (100–638, *E. coli* numbering; Brosius *et al.*, 1978), which showed maximum similarity, 99.4 % (536/539 nt), with the corresponding gene from the proposed type strain of '*B. ansorpii*' (GenBank accession no. AY594190). The species with the next highest similarities were *B. petrii*, 98.3 % (530/539 nt; GenBank accession no. AJ249861), and *B. hinzii*, 97.8 % (527/539; GenBank accession no. AF177667). Examination of the secondary structure of the sequenced region indicated four signature sequences, which appeared unique to '*B. ansorpii*' within the *Bordetella* genus (A, 134; U, 185; A, 192; A, 463; numbering with respect to *E. coli*). The *ompA* sequence of strain H050680373 was determined, and showed maximum similarity, 98.5 % (550/558 nt), within the coding sequence of the *ompA* gene from the '*B. ansorpii*' type strain (GenBank accession no. AY594191). The eight nucleotide differences comprised seven silent and one non-silent (proline instead of alanine) mutation compared to the type strain. The sequence with the next highest similarity was the *ompA* coding region of *B. pertussis* with a similarity of 86.9 % (477/549 nt). The *risA* sequence of strain H050680373, 568 nt, was determined, and showed maximum similarity, 96.7 % (550/568 nt), with the coding region of the *risA* gene from the '*B. ansorpii*' type strain (GenBank accession no. AJ242553). The sequences with the next highest similarities were from the same coding region in *B. petrii* (GenBank accession no. AJ242553) 91.9 % (384/418 nt), then *B. avium* (AJ224800), *B. bronchiseptica* (Z97065), *B. parapertussis* (AJ224800) and *B. pertussis* (AJ224798), all with similarities of 91.7 % (521/568 nt).

Discussion

In the case presented here, it is presumed that the long-term intravascular access line became colonized with the organisms described above, which contributed to the patient's rather non-specific general ill-health at the time of presentation in the clinic, and which resolved after line removal and appropriate antimicrobial therapy. The source of the '*B. ansorpii*' is unknown and there was nothing unusual in the patient history that is not indicated above; however, external contamination was considered unlikely. DNA sequence analysis of the genes above confirmed the identity of the isolate H050680373 as '*B. ansorpii*'. The original description of '*B. ansorpii*' sp. nov. was based on a single isolate, and to the best of our knowledge, no further isolates of this species have been reported from any source. At the time of writing, this species name is listed as without standing in nomenclature (http://www.bacterio.cict.fr/nonvalid.html), because on submission of the original article by Ko *et al.* (2005), the type strain had not been placed into two culture collections in two countries. To the best of our knowledge this has now been done.

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References

Andrews, J. M. (2001). The development of the BSAC standardized method of disc diffusion testing. *J Antimicrob Chemother* **48** (Suppl. 1), 29–42.

Brosius, J., Palmer, M. L., Kennedy, P. J. & Noller, H. F. (1978). Complete nucleotide sequence of a 16S ribosomal RNA gene from *Escherichia coli*. *Proc Natl Acad Sci U S A* **75**, 4801–4805.

Fry, N. K., Duncan, J., Malnick, H., Warner, M., Smith, A. J., Jackson, M. S. & Ayoub, A. (2005). *Bordetella petrii* clinical isolate. *Emerg Infect Dis* **11**, 1131–1133.

Ko, K. S., Peck, K. R., Oh, W. S., Lee, N. Y., Lee, J. H. & Song, J.-H. (2005). New species of *Bordetella, Bordetella ansorpii* sp. nov., isolated

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from the purulent exudate of an epidermal cyst. *J Clin Microbiol* **43**, 2516–2519.

Parton, R. (2005). *Bordetella*. In *Topley & Wilson's Microbiology and Microbial Infections*, pp. 1786–1817. Edited by S. P. Borriello, P. R. Murray & G. Funke. London: Hodder Arnold.

von Wintzingerode, F., Schattke, A., Siddiqui, R. A., Rosick, U., Göbel, U. B. & Gross, R. (2001). *Bordetella petrii* sp. nov., isolated from an anaerobic bioreactor, and emended description of the genus *Bordetella*. *Int J Syst Evol Microbiol* **51**, 1257–1265.

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