Case Report

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Bordetella petrii from a clinical sample in Australia: isolation and molecular identification

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The first isolation of *Bordetella petrii* from a patient with chronic suppurative mastoiditis is reported. Molecular characterization of the isolate was performed by sequencing the small-subunit rRNA gene, the *Bordetella* outer-membrane protein A gene (*ompA*) and the *RisA* response regulator gene (*risA*). This is the first reported case of *B. petrii* causing suppurative mastoiditis and only the second documented case of a clinically significant *B. petrii* isolate.

Case report

A 30-year-old Bangladeshi male presented to an ear, nose and throat surgeon with a 4-year history of chronic discharge from the right ear. He was first diagnosed with a cholesteatoma in Bangladesh in 2000 and underwent three operative procedures for clearance of the cholesteatoma and removal of mastoid air cells. However, chronic discharge from the ear continued. In 2004 he emigrated to Australia and was reviewed by an ear, nose and throat surgeon. He was reviewed regularly over the next 12 months with continuing discharge from the ear. In November 2005, a modified mastoidectomy and reconstruction were performed. Histopathology revealed granulation tissue, scar tissue and adipose tissue with fat necrosis and a mixed heavy acute and chronic inflammatory infiltrate with foci of acute suppurative inflammation.

A swab was sent to the Microbiology Department for routine microbiological analysis. Horse blood agar (HBA; Oxoid), MacConkey agar (MA; Oxoid) and chocolate agar (CHA; Oxoid) were inoculated and incubated for 48 h at 35 °C under 5 % CO₂ atmospheric conditions. A Sabouraud agar plate (Oxoid) was also inoculated and incubated at 30 °C in air for 7 days. A Gram stain was performed; this showed numerous polymorphs and predominant Gram-negative rods. After 24 h incubation, HBA, MA and CHA cultures demonstrated heavy growth of a Gram-negative bacillus. The Gram-negative bacillus was unable to be identified by routine laboratory protocols using standard biochemical reactions. An API 20NE (bioMérieux) was inoculated according to the manufacturer's recommendations and after 48 h incubation gave a profile number of 1000427 and an identification of *Alcaligenes denitrificans* with 88.6 % probability and an 'acceptable identification' confidence level. The isolate was also analysed on a

VITEK 2 Compact (bioMérieux). The bionumber obtained was 4000001101500200, giving an identification of *Pseudomonas fluorescens* with 97 % probability and an 'excellent identification' confidence level. Analysis of whole-cell long-chain fatty acids was performed using the Microbial Identification System (MIS; MIDI). The MIS identified this isolate as *Achromobacter xylosoxidans* subsp. *denitrificans* with a similarity index (SI) of 0.658. An SI index of 0.5 or greater is considered correct to the species level. Due to discrepancies between these identification systems, sequence analysis was performed on the isolate.

MICs were determined using the VITEK 2 Compact (bioMérieux). Susceptibility profiles are shown in Table 1.

Table 1. MICs for the *B. petrii* isolate

Antimicrobial agent	MIC
Ampicillin	≥ 32
Amoxicillin/clavulanic acid	4
Ticarcillin/clavulanic acid	≤ 8
Piperacillin/tazobactam	≤ 4
Cefalotin	≥ 64
Cefazolin	≥ 64
Cefoxitin	≥ 64
Ceftazidime	16
Ceftriaxone	≥ 64
Cefepime	≥ 64
Meropenem	≤ 0.25
Amikacin	8
Gentamicin	4
Tobramycin	2
Nalidixic acid	≥ 32
Ciprofloxacin	2
Norfloxacin	8
Trimethoprim	≥ 16

The GenBank/EMBL/DDBJ accession numbers for the sequences described in this paper are EF212440, EF368181 and EF368182.

Genomic DNA was extracted using a QIAamp DNA Mini kit (QIAGEN) and DNA amplification of the small-subunit rRNA gene was performed using universal primers as previously described by Fry *et al.* (2005). Amplification of the genes for the *Bordetella* outer-membrane protein A (*ompA*) and the *RisA* response regulator (*risA*) was undertaken according to the method of von Wintzingerode *et al.* (2001). The PCR products were purified using the QIAquick PCR Purification kit (QIAGEN) according to the manufacturer's instructions. The PCR products were sequenced in both directions on an ABI Prism 3730 automated sequencer at the SUPAMAC facility (Royal Prince Alfred Hospital, Sydney). The sequences generated were compared to those available in the GenBank databases using the BLASTN program run on the National Center for Biotechnology Information server (<http://www.ncbi.nlm.nih.gov/blast/>).

The sequence data generated from the three different gene loci provided a conclusive identification of *Bordetella pertussis*. The 16S rRNA gene sequence demonstrated 100% homology with the 16S rRNA gene sequence of the first reported clinical isolate of *B. pertussis*, the GDH030510 strain (GenBank accession no. AJ870969) (Fry *et al.*, 2005). The next highest homology of 99% was with the prototype environmental isolate, the DSM 12804^T strain of *B. pertussis* (GenBank accession no. AJ249861) (von Wintzingerode *et al.*, 2001). The *risA* and the *ompA* gene sequences demonstrated 100% homology with both the *risA* and the *ompA* gene sequences of the GDH030510 strain of *B. pertussis* (GenBank accession nos AJ920265 and AJ920264, respectively).

Discussion

The family *Alcaligenaceae* comprises the genera *Bordetella* and *Alcaligenes* and based on phenotypic and genotypic data also includes the genus *Achromobacter* (Yabuuchi *et al.*, 1998). The genus *Bordetella* currently consists of nine species. *Bordetella pertussis* is the aetiological agent of whooping cough (Hewlett, 1995). *Bordetella parapertussis* causes a milder respiratory infection in humans and is also a causative agent of chronic pneumonia in sheep (Hewlett, 1995). *Bordetella holmesii* has been isolated on a number of occasions from blood and sputum of humans (Weyant *et al.*, 1995; Tang *et al.*, 1998; Mazengia *et al.*, 2000). *Bordetella trematum* has been shown to cause both ear and wound infections exclusively in humans (Vandamme *et al.*, 1996). *Bordetella ansorpii* was identified recently when it was isolated from the purulent exudate of a patient with an epidermal cyst (Ko *et al.*, 2005). *Bordetella bronchiseptica* causes respiratory tract infections in many mammals and only rarely infects humans (Woolfrey & Moody, 1991; Gueirard *et al.*, 1994). *Bordetella hinzii* has been isolated from immunocompromised human hosts, but is mainly a commensal of the respiratory tract of poultry (Cookson *et al.*, 1994; Vandamme *et al.*, 1995; Funke *et al.*, 1996). *Bordetella avium*, a bird pathogen, is the only *Bordetella* species that has never been found in humans (Arp & Cheville, 1984). *B. pertussis* was first isolated and identified

from an environmental source (von Wintzingerode *et al.*, 2001). There has only been one previously reported clinical case of *B. pertussis* infection causing mandibular osteomyelitis (Fry *et al.*, 2005).

The sequence data generated from the 16S rRNA, *risA* and *ompA* genes definitively identified the isolate as *B. pertussis*. The isolate was more closely related to the previously reported clinical isolate described by Fry *et al.* (2005) from London than to the type strain environmental isolate (von Wintzingerode *et al.*, 2001). Our isolate demonstrated 100% homology with the 16S rRNA, *risA* and *ompA* genes of the first clinical isolate (Fry *et al.*, 2005) and 99% homology (reflecting a 12 bp difference) with the 16S rRNA gene of the *B. pertussis* prototype strain, DSM 12804^T. There were some phenotypic differences between our isolate and the other reported *B. pertussis* strains, DSM 12804^T and GDH030510. Our isolate failed to grow under anaerobic conditions and demonstrated motility whilst both other strains were facultatively anaerobic and non-motile. The Australian *B. pertussis* strain assimilated phenylacetate but not malate, while the environmental DSM 12804^T strain did not assimilate phenylacetate but did assimilate malate.

The patient was treated with clarithromycin 500 mg b.d. for a period of 2 months. At follow-up appointments after antimicrobial drug therapy had ceased, clinical findings were normal with no signs of infection, and with significant improvement in hearing reported by the patient. The successful clinical outcome highlights the positive response to antimicrobial treatment.

The source of infection is unknown, but as *B. pertussis* has been isolated from river sediment (von Wintzingerode *et al.*, 2001), it is likely that the isolate originated from an environmental source, possibly in Bangladesh given the patient's history of residence in that country. The pathogenesis of *B. pertussis* is currently unknown. The characterization and identification of this clinical isolate may aid in the understanding and elucidation of the pathogenic potential of this unique member of the genus *Bordetella*.

This report describes the second clinical isolate of *B. pertussis* worldwide and the first reported isolation of this organism from the mastoid and from a patient in Australia.

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