Bordetella petrii
Clinical Isolate
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We describe the first clinical isolate of Bordetella petrii from a patient with mandibular osteomyelitis. The only previously documented isolation of B. petrii occurred after the initial culture of a single strain from an environmental source.

A 67-year-old man visited an emergency dental clinic, where he complained of toothache in the lower right mandibular quadrant. Examination showed a root-filled lower right canine tooth that was mobile and tender to percussion. The tooth was extracted uneventfully under local anesthesia. The patient returned after several days with pain at the extraction site. A localized alveolar osteitis was diagnosed, and local debridement measures were instituted. These measures proved unsuccessful, and repeat examination showed submandibular lymphadenopathy and tenderness to palpation in the buccal sulcus in the extraction site. Radiographs showed no abnormality at this stage.

A course of oral amoxicillin (250 mg, every 8 h for 5 days) followed by oral metronidazole (200 mg every 8 h for 5 days) was prescribed. Symptoms persisted, with increasing severity of pain in the affected area, and the patient was referred to a tertiary referral center. On examination there, the patient had normal full blood count, hematinics, and glucose levels. Ultrasound examination of the submental soft tissue region did not indicate any abnormal pathology. Radiographs showed radiolucencies in the bone surrounding the extraction site. During this period a 3-week course of oral co-amoxiclav (375 mg, every 8 h) was prescribed. A bone biopsy was performed under local anesthesia, and a diagnosis of mandibular osteomyelitis was made.

A portion of the bone biopsy specimen was cultured in 10 mL Fastidious Anaerobe Broth (FAB) (BioConnections, Wetherby, UK) and incubated for 48 h at 37°C in air. After 48 h, the FAB was subcultured onto 1) Fastidious Anaerobe Agar (FAA, BioConnections), incubated for 72 h at 37°C under anaerobic conditions, and 2) Columbia Blood Agar (CBA, BioConnections), incubated for 72 h at 37°C under 5% CO₂ atmospheric conditions. Culture on both FAA and CBA showed a pure growth of a facultative gram-negative bacillus that had not been identified with routine laboratory protocols. Initial susceptibility testing using disk diffusion indicated apparent susceptibility of the isolate to erythromycin, gentamicin, ceftriaxone, and piperacillin/tazobactam. The isolate was resistant to amoxicillin, co-amoxiclav, tetracycline, clindamycin, ciprofloxacin, and metronidazole. After initial sensitivity results, a 6-week course of oral clarithromycin (500 mg, 8 hourly) was begun.

At follow-up appointments 3 months and 6 months after antimicrobial drug therapy ceased, clinical and radiographic findings were not unusual, and the infected area healed successfully. Despite the successful clinical outcome, the isolate was subsequently shown to be resistant to clarithromycin in vitro (Table). Improvement of the osteomyelitis may also have been facilitated by the biopsy procedure, during which a sequestrum of bone was removed.

The gram-negative bacillus (designated strain GDH030510) was submitted to the Health Protection Agency, Centre for Infections, London, for identification. Preliminary tests results were consistent with those described for members of the genus Bordetella. Colonies had the following phenotypic characteristics: positive reaction for oxidase and negative reaction for urease production, motility using the hanging-drop method at 37°C, and slide agglutination with B. pertussis and B. parapertussis antisera (Difco, Shannon, Ireland). The organism could be cultured on MacConkey agar and was non-hemolytic on blood agar. Genomic DNA was extracted by using the InstaGene Purification Matrix (BioRad, Hercules, CA, USA). DNA amplification of small-subunit (SSU) rRNA genes was performed by using primers 27f and 1525r (1). Amplification and sequencing of the gene for the Bordetella outer membrane protein A (ompA) and the RisA response regulator (risA) were as described by von Wintzingerode et al. (2). Reaction mixes contained the following: 2 mmol/L MgCl₂, 10 mmol/L Tris-HCl (pH 8.3), 50 mmol/L KCl, 0.05% W-1 (Invitrogen, Paisley, UK), 0.2 µmol/L of each deoxynucleotide (Roche Applied Science, Lewes, UK) 20 pmol of each primer (MWG Biotech, Milton Keynes, UK), 2.5 U of Taq DNA polymerase (Invitrogen), 1.0 mol/L betaine (Sigma-Aldrich, Gillingham, UK), and 10 µL template DNA. Amplification was performed in a DNA Engine (MJ Research, Bio-Rad) by using 35 cycles of denaturation of 1 min at 94°C, 1 min at 55°C, and 2 min at 72°C and a final step at 72°C for 3 min. Amplicons from triplicate samples were pooled and purified with Montage PCR96 filter plates (Millipore, Watford, UK). For the rRNA gene, nucleotide sequence determination was done by using the primers used for amplification, together with internal primers (1). Sequencing was performed with the Dye Terminator Cycle Sequencing kit (Beckman Coulter, High Wycombe, UK) and analyzed on

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The nucleotide sequences of the 16S rRNA gene, risA gene, and ompA gene of strain GDH030510 have been submitted to the EMBL Nucleotide Sequence Database under accession numbers AJ870969, AJ920265, and AJ920264, respectively. The 1,486 nucleotides (nt) of the SSU rRNA gene sequence that were determined showed a maximum similarity of 99.5% (1,468/1,476 nt). The 445 nt of the risA gene sequence of strain GDH030510 that were determined showed a maximum similarity value of 93.9% (418/445 nt) with the risA gene from the B. petrii type strain (AJ242553). The species with the next highest similarities were B. parapertussis, B. avium, B. bronchiseptica, and B. pertussis, all with similarities of 88.3% (393/445 nt). The 414 nt of the ompA gene sequence that were determined showed maximum a similarity value of 92.0% (381/414 nt) with the ompA gene from the B. petrii type strain (AJ242599). The species with the next highest similarities were B. bronchiseptica and B. parapertussis, both with similarities of 87.9% (364/414 nt).

Further susceptibility testing was undertaken, with MICs determined by agar dilution on diagnostic sensitivity test agar (Oxoid, Basingstoke, UK) supplemented with 5% lysed horse blood, with inocula equivalent to 0.5 and 2 McFarland standards, and with incubation at 37°C in air for 24 to 36 h to ensure adequate growth. No inoculum effects were noted on MICs for any antimicrobial agents. MICs are shown in the Table.

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**Table. Results of antimicrobial drug–susceptibility testing for strain GDH030510**

| Antimicrobial agent | MIC (µg/mL) |
|---------------------|-------------|
| Penicillin          | >32         |
| Ampicillin          | >256        |
| Piperacillin/tazobactam | 2         |
| Ceftriaxone         | >32         |
| Cefotaxime          | >32         |
| Ceftazidime         | 32          |
| Imipenem            | >32         |
| Meropenem           | >32         |
| Ertapenem           | >32         |
| Amikacin            | >256        |
| Gentamicin          | 4           |
| Tobramycin          | 16          |
| Ciprofloxacin       | >32         |
| Erythromycin        | 128         |
| Azithromycin        | 4           |
| Clarithromycin      | 64          |
| Clindamycin         | >256        |
| Chloramphenicol     | >256        |
| Cotrimoxazole       | 8           |
| Rifampin            | >32         |
| Tetracycline        | >256        |

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According to the manufacturer’s instructions, Contig assembly and sequence analyses were performed with Kodon, version 2.0 (Applied Maths, Kortrijk, Belgium) and BioNumerics, version 3.5 (Applied Maths). Consensus sequence was compared with public databases with the BLASTn program (http://www.ncbi.nlm.nih.gov/BLAST) (3), and sequences with the greatest similarity were downloaded for further analysis.

The next highest similarities were B. parapertussis, 98.4% (1,455/1,479 nt), and B. bronchiseptica, 98.3% (1,454/1,479 nt). The secondary structure of the SSU rRNA from both strain GDH030510 and the submitted sequence for the type strain of B. petrii (GenBank accession no. AJ249861) for all sequence analyses, regions not present in all sequences and ambiguous bases were excluded. The species with the next highest similarities were B. parapertussis, 98.4% (1,455/1,479 nt), and B. bronchiseptica, 98.3% (1,454/1,479 nt). The secondary structure of the SSU rRNA from both strain GDH030510 and the submitted sequence for the type strain of B. petrii were compared to that proposed for bacteria (4). Where possible (i.e., in stems), supportive evidence from base-pairing was sought. This analysis indicated that the assigned nucleotides at 3 separate locations in the GenBank B. petrii sequence were not supported, as they formed noncanonical base-pairing (C-U vs. C-G [156, 165], U-U vs. A-U [824, 875], A-C vs. G-C [838, 848]; *Escherichia coli* numbering [5]). Exclusion of these 3 bases increased the similarity to 99.5% (1,468/1,476 nt). The 445 nt of the risA gene sequence of strain GDH030510 that were determined showed a maximum similarity value of 93.9% (418/445 nt) with the risA gene from the B. petrii type strain (AJ242553). The species with the next highest similarities were B. parapertussis, B. avium, B. bronchiseptica, and B. pertussis, all with similarities of 88.3% (393/445 nt). The 414 nt of the ompA gene sequence that were determined showed maximum a similarity value of 92.0% (381/414 nt) with the ompA gene from the B. petrii type strain (AJ242599). The species with the next highest similarities were B. bronchiseptica and B. parapertussis, both with similarities of 87.9% (364/414 nt).
isolates of this species have been previously reported from any source.

The source of infection of the strain described here and the pathogenic role of *B. petrii* are currently unknown. However, the identification and characterization of further clinical isolates should help determine the reservoir and virulence potential of this intriguing species.

**Addendum**

A clinical isolate of a new species of *Bordetella*, *Bordetella ansorpii* sp. nov, has recently been reported (14). Phylogenetically, it appears to be more closely related to *B. petrii* than to other members of the genus.

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Dr. Fry is a principal clinical scientist in the Respiratory and Systemic Infection Laboratory, Centre for Infections, London. His interests include the detection, diagnosis, epidemiologic typing, and analysis of virulence factors of microorganisms, in particular, those belonging to the genera *Bordetella*, *Legionella*, and *Bartonella*.

**References**

1. Lane DJ. 16/23S rRNA sequencing. In: Stackebrandt E, Goodfellow M, editors. Nucleic acid techniques in bacterial systematics. Chichester (UK): John Wiley & Sons; 1991. p. 115–75.
2. von Wintzingerode F, Schattke A, Siddiqui RA, Rösick U, Göbel UB, Gross R. *Bordetella petrii* sp. nov., isolated from an anaerobic bioreactor, and emended description of the genus *Bordetella*. Int J Syst Evol Microbiol. 2001;51:1257–65.
3. McGinnis S, Madden TL. BLAST: at the core of a powerful and diverse set of sequence analysis tools. Nucleic Acids Res. 2004;32:W20–5.
4. Cannone JJ, Subramanian S, Schnare MN, Collett JR, D’Souza LM, Du Y, et al. The Comparative RNA Web (CRW) Site: an online database of comparative sequence and structure information for ribosomal, intron, and other RNAs. BMC bioinformatics [electronic resource] 2002;3:2. [cited 7 Jan 2005]. Available from http://www.biomedcentral.com/1471-2105/3/2
5. Brosius J, Ullrich A, Raker MA, Gray A, Dull TJ, Gutell RR, et al. Construction and fine mapping of recombinant plasmids containing the *rrnB* ribosomal RNA operon of *E. coli*. Plasmid. 1981;6:112–8.
6. Gerlach G, von Wintzingerode F, Middendorf B, Gross R. Evolutionary trends in the genus *Bordetella*. Microbes Infect. 2001;3:61–72.
7. Vandamme P, Heyndrickx M, Vancanneyt M, Hoste B, De Vos P, Falsen E, et al. *Bordetella trematuni* sp. nov., isolated from wounds and ear infections in humans, and reassessment of *Acaligenes denitrificans* Rüger and Tan 1983. Int J Syst Bacteriol. 1996;46:849–58.
8. Vandamme P, Hommez J, Vancanneyt M, Monsieurs M, Hoste B, Cookson B, et al. *Bordetella hinzii* sp. nov., isolated from poultry and humans. Int J Syst Bacteriol. 1995;45:37–45.
9. Weyant RS, Hollis DG, Weaver RE, Amin MFM, Steigerwalt AG, O’Connor SP, et al. *Bordetella holmesii* sp. nov., a new gram-negative species associated with septicemia. J Clin Microbiol. 1995;33:1–7.
10. Funke G, Hess T, von Graevenitz A, Vandamme P. Characteristics of *Bordetella hinzii* strains isolated from a cystic fibrosis patient over a 3-year period. J Clin Microbiol. 1996;34:966–9.
11. Kattar MM, Chavez JF, Limaye AP, Rassoulian-Barrett SL, Yarfitz SL, Carlson LC, et al. Application of 16S rRNA gene sequencing to identify *Bordetella hinzii* as the causative agent of fatal septicemia. J Clin Microbiol. 2000;38:789–94.
12. Mazengia E, Silva EA, Peppe JA, Timperi R, George H. Recovery of *Bordetella holmesii* from patients with pertussis-like symptoms: use of pulsed-field gel electrophoresis to characterize circulating strains. J Clin Microbiol. 2000;38:2330–3.
13. Yih WK, Silva EA, Ida J, Harrington N, Lett SM, George H. *Bordetella holmesii*-like organisms isolated from Massachusetts patients with pertussis-like symptoms. Emerg Infect Dis. 1999;5:441–3.
14. Ko KS, Peck KR, Oh WS, Lee NY, Lee JH, Song JH. New species of *Bordetella*, *Bordetella ansorpii* sp. nov., isolated from the purulent exudate of an epidermal cyst. J Clin Microbiol 2005;43:2516–9.

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