

# Identification by 16S rRNA Gene Sequencing of an *Actinomyces hongkongensis* Isolate Recovered from a Patient with Pelvic Actinomycosis

A. N. Flynn,<sup>c</sup> C. A. Lyndon,<sup>d</sup> D. L. Church<sup>a,b,c</sup>

Calgary Laboratory Services<sup>a</sup> and Departments of Pathology and Laboratory Medicine,<sup>b</sup> Medicine,<sup>c</sup> and Obstetrics and Gynecology,<sup>d</sup> University of Calgary, Calgary, Alberta, Canada

**A case of *Actinomyces hongkongensis* pelvic actinomycosis in an adult woman is described. Conventional phenotypic tests failed to identify the Gram-positive bacillus isolated from a fluid aspirate of a pelvic abscess. The bacterium was identified by 16S rRNA gene sequencing and analysis using the SmartGene Integrated Database Network System software.**

A 47-year-old, gravida 2, para 2, immunocompetent woman presented to the hospital with pelvic pain and fever 13 days after a total abdominal hysterectomy and salpingectomy for menorrhagia and hydrosalpinx. Her medical profile included smoking, perforated gastric ulcer, appendectomy, tubal ligation reversal, and two diagnostic laparoscopies. She had no history of intrauterine contraceptive device (IUCD) use. One gram of intravenous (i.v.) prophylactic cefazolin had been administered before the procedure. The surgery had proceeded without complication (200-ml estimated blood loss), and the patient was discharged on postoperative day 3. On presentation, the patient's white blood cell count was  $21.7 \times 10^9$  cells/liter ( $18.6 \times 10^9$  neutrophils/liter), the hemoglobin level was 117 g/liter, and the platelet count was  $570 \times 10^9$  platelets/liter. Electrolyte and creatinine levels were normal. A mass was palpated at the vaginal apex, and computed tomography (CT) showed a bilocular abscess immediately above the vaginal vault (the larger lobule measuring 8.8 by 5.8 cm), with regional inflammation (Fig. 1). The patient received empirical cefazolin and metronidazole, followed by clindamycin and gentamicin. She was then taken to the operating room for incision and drainage via a vaginal approach, but the mass was no longer palpable, and the procedure was aborted. Her antibiotic regimen was changed to ampicillin, gentamicin, and metronidazole, and fluid was drained from the residual mass under CT guidance the following day. Final CT imaging demonstrated a marked decrease in the size of the abscess. The patient improved clinically and was discharged home after 4 days in the hospital. As she had been afebrile for 2 days, no oral antibiotics were prescribed upon discharge. The patient remains free of symptoms 6 years after discharge from the hospital. The conjoint ethics board at the University of Calgary approved this study.

The fluid aspirate was subcultured on Columbia sheep blood agar (BA), chocolate agar (CHOC), MacConkey agar, and Brucella blood agar (BBA) (PML Microbiologicals, Wilsonville, OR) plates and incubated anaerobically at 35°C for 48 h before examination using an Anoxomat Mark II system (Mark Microbiology, Drachten, Netherlands). Growth occurred on BBA plates as non-hemolytic, pinpoint colonies. Gram staining showed the isolate to be a straight Gram-positive bacillus. Biochemical analysis using the Vitek 2 ANC card (bioMérieux, Laval, Quebec, Canada) provided a low-discrimination organism split between *Actinomyces meyeri* and *Propionibacterium acnes*. The isolate was biochemically inert, except for positive arginine dihydrolase, alkaline phosphatase,

tyrosine arylamidase, leucine arylamidase, phenylalanine arylamidase, and proline arylamidase reactions. The isolate was nonmotile and did not produce catalase or indole. Metabolic end products were not tested. Vitek matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) (bioMérieux) analysis gave a 99% coefficient of variation (CV) for identification as *Microbacterium testaceum*.

Molecular identification was done by fast partial sequencing of the 16S rRNA gene (523 bp) with MicroSeq 500 kits and an ABI Prism 3130 sequencer (Applied Biosystems, Foster City, CA), using standard methods (1–3). A BLAST search against the SmartGene Integrated Database Network System (IDNS) Bacteria database indicated that the species most closely related to the pelvic isolate was *Actinomyces hongkongensis*, and the overall identity score was 100%, with no mismatches (4, 5). The references were from a recently described species, *Actinomyces hongkongensis* (strain HKU8<sup>T</sup>), within the *Actinomycetales* order, *Actinomycineae* suborder, and *Actinomyces* genus (6), which was recently isolated from another case of pelvic actinomycosis (5). Our isolate also showed good identity (>90%) with other members of the genus *Actinomyces* (6). The most closely related known species within GenBank was *Actinomyces marimammalium*, with a sequence identity of 93%, while other *Actinomyces* species sequences within the IDNS database gave slightly lower identities (87 to 91%) (Fig. 2). Alignment of the partial 16S rRNA sequence from our clinical isolate and *A. hongkongensis* strain HKU8<sup>T</sup> showed 100% identity of 16S rRNA sequences using CLUSTALW2 (<http://www.ebi.ac.uk/Tools/clustalw2/index.html>) (7). Phylogenetic analysis was done by using MEGA 5.1 software and the neighbor-joining method (2, 8) and gave a bootstrap score of 100 between our pelvic isolate and the sequences from *A. hongkongensis* strain HKU8<sup>T</sup>, while other *Actinomyces* species were related but had much lower bootstrap scores (Fig. 2).

Antibiotic susceptibility testing using Etest strips (bioMérieux)

Received 21 February 2013 Returned for modification 19 March 2013

Accepted 15 May 2013

Published ahead of print 22 May 2013

Address correspondence to D. L. Church, [Deirdre.church@cls.ab.ca](mailto:Deirdre.church@cls.ab.ca).

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doi:10.1128/JCM.00509-13



FIG 1 Axial computed tomography scan of the pelvis demonstrating a bilocular perivaginal abscess (arrows).

showed the isolate to be sensitive to penicillin (MIC = 0.2 µg/ml) and clindamycin (MIC < 4 µg/ml) but resistant to metronidazole (MIC > 256 µg/ml).

This report demonstrates that partial or complete sequencing of the 16S rRNA gene is a valuable tool for definitive molecular

identification of important clinical isolates that cannot be readily identified by phenotypic methods (1, 3–5). *Actinomyces* species are primarily anaerobic, filamentous, Gram-positive, opportunistic bacteria that colonize human mucous membranes. There are over 40 currently recognized species of *Actinomyces* that are morphologically similar and resist identification by traditional phenotypic tests (9). Therefore, sequencing of the 16S rRNA gene has become a useful tool for the identification of members of this genus to the species level, not only to definitively identify clinically relevant isolates but also to improve our understanding of the role of novel *Actinomyces* spp. in causing specific types of clinical infections (1, 3). In 2003, 16S rRNA sequencing was used to identify a novel species, *A. hongkongensis* strain HKU8<sup>T</sup>, isolated from the salpinx of a woman presenting with IUCD-associated pelvic actinomycosis (5). Our isolate demonstrated phenotypic characteristics similar to those previously described for the HKU8<sup>T</sup> isolate, and fast partial sequencing of the 16S rRNA gene demonstrated complete molecular identity between our strain and HKU8<sup>T</sup> (GenBank accession no. AF433168) (5). Although *Actinomyces israelii* has been most frequently described in cases of pelvic actinomycosis (9), many other species have been implicated in human disease (6, 10, 11). Our report is the second to document the

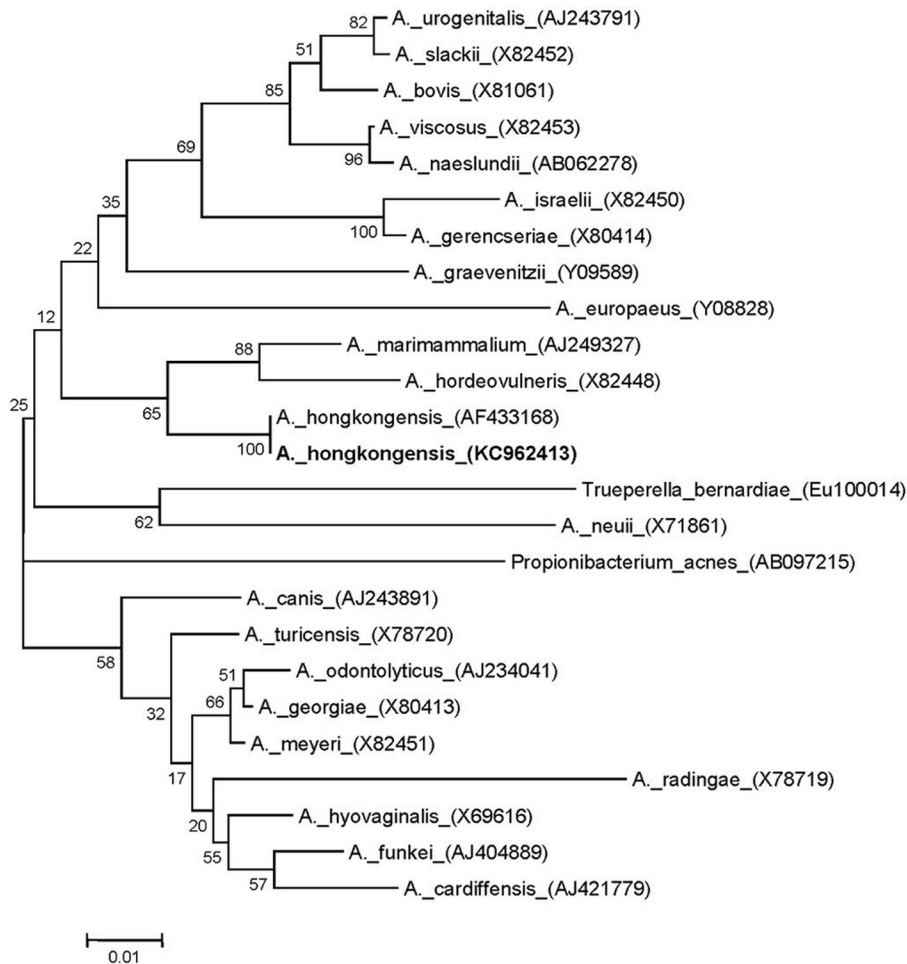


FIG 2 Neighbor-joining tree of 523 bp of the 16S rRNA gene sequences of the pelvic abscess isolate (GenBank accession no. KC962413) and *Actinomyces* reference strains. Branch support is recorded at the nodes as a percentage of 1,000 bootstrap iterations. GenBank accession numbers are shown in parentheses.

isolation of *A. hongkongensis* and confirms its role in causing pelvic actinomycosis. Due to the rarity of this species, it is currently unknown whether *A. hongkongensis* has a propensity for localizing to the abdominopelvic region.

We could not determine whether *A. hongkongensis* was the primary pathogen or part of a polymicrobial flora in this abdominopelvic abscess because the patient received broad-spectrum antibiotics prior to aspiration and culture. Typically, *Actinomyces* spp. are recovered as a component of mixed bacterial flora in complex abscess formation in various locations. The most commonly affected sites of actinomycosis are cervicofacial (50%), abdominopelvic (20%), and thorax (15%) (9). Abdominopelvic actinomycosis generally occurs in association with abdominal surgery, appendicitis, ruptured viscus, neoplasia, or the prolonged use of an IUCD (12). Progression is usually indolent and leads to nonspecific symptoms, such as intermittent abdominal pain, low-grade fever, and weight loss. While CT imaging can localize the lesion, findings are usually nondiagnostic. For these reasons, abdominopelvic actinomycosis is often diagnosed following laparotomy for a suspected neoplasm. On presentation, the patient described here was thought to have a postoperative bacterial abscess, but actinomycosis was not specifically suspected. The final diagnosis of actinomycosis is usually based upon histological confirmation of actinomycotic sulfur granules or positive cultures, both of which are insensitive tests (9).

The treatment of actinomycosis has traditionally taken the form of extended courses of antibiotics, typically intravenous penicillin G for 2 to 6 weeks followed by oral penicillin V for 6 to 12 months (9). However, with adequate source control, substantially shorter antibiotic regimens have proven effective for abdominopelvic actinomycosis. For example, Atad et al. described a patient cured after only 2 days of effective antibiotics following salpingo-oophorectomy for adnexal actinomycosis (13). The outcome of our patient agrees with this therapeutic strategy and suggests that extended antibiotic courses may not be required. Furthermore, total excision of actinomycotic collections may be unnecessarily invasive if image-guided percutaneous drainage is a viable option. Further studies are required to determine which *Actinomyces* species and anatomical sites of infection would most benefit from percutaneous drainage and short courses of antibiotics.

In summary, clinical microbiology laboratories should be aware that *A. hongkongensis* may be a human pathogen in pelvic infections in women. Isolates meeting the preliminary phenotypic

characteristics of *Actinomyces* spp. recovered from pelvic or abdominal abscesses should be referred for definitive identification using partial 16S rRNA gene sequencing. Delineation of the clinical significance and pathogenic potential of *A. hongkongensis* in humans is dependent upon further isolation from clinical samples and full phenotypic and genotypic characterization.

**Nucleotide sequence accession number.** The sequence of the strain described here has been deposited in GenBank under accession number [KC962413](https://www.ncbi.nlm.nih.gov/nucl/10.1136/bmj.d6099).

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