**Campylobacter fetus** of Reptile Origin as a Human Pathogen

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A *Campylobacter* species was isolated from blood from a febrile patient with precursor T-cell acute lymphoblastic leukemia, and after antibiotic treatment, a similar bacterium was isolated from blood 37 days later. Although phenotypic testing did not definitively identify the organisms, molecular analysis indicated that they were the same strain of *Campylobacter fetus* subsp. *fetus* and were of reptile origin.

**CASE REPORT**

A 20-year-old man presented to the hospital with a productive cough for 2 weeks and fever of 40.6°C for 1 day. Seven months earlier, a computerized tomographic scan of the chest showed a 7-cm-diameter mediastinal mass compressing the trachea. Peripheral blood flow cytometry at that time was diagnostic of precursor T-cell acute lymphoblastic leukemia. Because of pneumocystis pneumonia, which was treated, the patient then received trimethoprim-sulfamethoxazole prophylaxis three times a week on a continuing basis. Numerous courses of chemotherapy led to neutropenia, complicated by *Candida tropicalis* fungemia and then *Streptococcus mitis* bacteremia, which both resolved with treatment. Two weeks prior to admission, the patient received chemotherapy with methotrexate and 6-mercaptopurine.

On the day the patient was admitted to the hospital, he reported worsening cough, fever, and epigastric pain. On examination, he appeared to be moderately ill with a temperature of 39.4°C, pulse of 110 beats/min, blood pressure of 110/48 mm Hg, and respiratory rate of 14 breaths/min; his physical examination was otherwise normal. The leukocyte count was 3,900/mm<sup>3</sup>, with an absolute neutrophil count of 2,650/mm<sup>3</sup> and a platelet count of 52,000/mm<sup>3</sup>. He had 6.6 g of hemoglobin per dl. The chest X-ray was normal. After cultures were obtained, he was given cefepime intravenously. Two days after admission, one of four blood culture bottles was reported as obtained, he was given cefepime intravenously. Two days after admission, one of four bottles was reported as obtained, he was given cefepime intravenously. Twelve days after admission, one of four bottles was reported as obtained, he was given cefepime intravenously. Two days after admission, one of four bottles was reported as obtained, he was given cefepime intravenously. Twelve days after admission to the hospital, the patient received chemotherapy with methotrexate and 6-mercaptopurine. Cefepime was changed to imipenem-cilastatin. He also was noted to have erythema and tenderness of the right arm in an area near a prior phlebotomy site. Vancomycin was added for presumed cellulitis and was continued for 1 week, with complete resolution of erythema. The chest X-ray was normal. After cultures were obtained, the patient recovered and was readmitted 3 weeks later for elective chemotherapy with cytarabine and etoposide. Four days after admission to the hospital, he developed abdominal pain and a temperature of 38.9°C. The chest X-ray was normal. After blood cultures were obtained, the patient was empirically treated with cefepime and metronidazole, which was changed 4 days later to intravenous levofloxacin and metronidazole. The absolute neutrophil count at that time was 11,300/mm<sup>3</sup>, which subsequently decreased to a nadir of <50/mm<sup>3</sup> 7 days after admission to the hospital. Abdominal tomography revealed panniculitis. Stool culture and stool *Clostridium difficile* toxin assays were negative. Eight days after admission to the hospital, the patient had improved and was discharged on a 10-day regimen of levofloxacin and metronidazole. Subsequently, the blood cultures obtained on admission again grew a *Campylobacter* species in one of four bottles, susceptible again to levofloxacin and tetracycline. On the basis of the recurrent *Campylobacter* isolations, levofloxacin then was continued prophylactically for the duration of subsequent chemotherapies.

*Campylobacter fetus* is a gram-negative, slender, spiral, bacterial pathogen that may cause enteritis, abortion, bacteremia, endocarditis, or meningitis in humans (4, 15). *C. fetus* may be either type A or type B based on serotype, lipopolysaccharide structure, and surface layer protein (SLP) type (5, 9, 12). The *C. fetus* species is currently divided into *C. fetus* subsp. *fetus* and *C. fetus* subsp. *venerealis*. *C. fetus* has been isolated from ungulates, swine, humans, birds, and reptiles (7, 14). In 1985, Harvey and Greenwood reported the isolation of *C. fetus* from turtles owned by an ill child (7). Methods to distinguish *C. fetus* strains of mammal and reptile origin have been published recently (16). We now report the first confirmed isolation from a human of *C. fetus* with markers of reptile origin. In this case, the patient was symptomatic due to recurrent bacteremia with this organism.

**Microbiology.** Growth of both isolates on blood agar plates occurred at 37 and 42°C using a CampyPak envelope (Becton Dickinson, Sparks, Md.) to create microaerobic conditions. The organisms were gram negative, curved, catalase positive, oxidase positive, H<sub>2</sub>S producing, resistant to nalidixic acid and cephalothin, and unable to hydrolyze hippurate. Antibiotic susceptibility was determined using E Test (AB Biodisk North...
From both strains isolated, the expected fetus sapD performed with primers SDF01 and SDR01, specific for C. fetus strain 23D (Fig. 1B and C). Since SLPs have been shown to be the major C. fetus virulence factors (3, 6), strains 03-427 and 03-445 were analyzed for SLP expression using a polyclonal antibody raised against the 97-kDa major SLP of type A strain 82-40LP (16) as described previously (18). The immunoblot analysis showed that each strain expressed a 97-kDa protein as its major SLP (Fig. 2).

Sequence analyses. The identity of isolate 03-427 was subsequently determined by sequence analysis of 16S rRNA (16). PCR was performed, using universal 16S ribosomal DNA primers 8F (5'-AGAGTTTGATYMTGGCTCAG) and 1510R (5'-TACGGYTACCTTGTTACGACTT) (11), and the products were sequenced. Using the Genetics Computer Group Gap program, the sequence was compared with all known C. fetus 16S rRNA sequences; it showed 100% identity with the sequence of reptile C. fetus strain 85-388 (GenBank accession number AY621302) (16). To further confirm that these isolates originated in a reptile, we examined the sapD sequence, since it shows greater variation between reptile and mammalian strains than does 16S rRNA (16). The sapD PCR product was amplified using primers SDF01 and SDR01 (16), and sequence analysis showed 100% homology with sapD from C. fetus reptile strain 85-388 gene (GenBank accession number AY621300) (16) and 46 mismatches with sapD from mammalian hosts (16).

Further molecular characterization of strains. On the basis of our previous study, reptile C. fetus strains may possess a 187-bp noncoding DNA insertion near the upstream boundary of the sap island. To test this property in the two strains isolated, we performed PCR using the 187-bp insertion-specific primers IF and IR (18). The results indicated that the strains isolated each possess a positive band (Fig. 1D). Sequence analysis of the PCR product amplified from strain 03-427 showed a 9-bp (ATTTATTTA) deletion at bp 53 of the amplified 149-bp product in strain 85-388 (18) and seven other single-nucleotide polymorphisms compared with the sequence of strain 85-388 (data not shown).

Randomly amplified polymorphic DNA (RAPD) can be used to distinguish the mammalian and reptilian C. fetus strains (Z. Tu and M. J. Blaser, unpublished data). Analysis with RAPD and primer 1254 (1) showed that the two isolated C. fetus strains produce 1.2-, 1.6-, and 2.5-kb bands, consistent with analysis of reptile C. fetus strain 85-388, whereas the two mammalian strains 23D (type A) and 84-107 (type B) produce 2.5- and 3.0-kb bands, as expected (Fig. 1E).

C. fetus isolates from reptiles differ from C. fetus subsp. fetus and C. fetus subsp. venerealis based on the results from 16S rRNA, recA, and sapD sequence analyses and may be a new Campylobacter species or subspecies (16). A C. fetus strain isolated from a turtle was suggested to be the cause of an acute diarrheal illness in a human, but there was no isolate from the affected child (7).

Genotypic approaches utilizing PCRs specific for sapA, sapB, reptile sap island insertion, and RAPD, as well as sapD and 16S rRNA molecular sequencing methods, allowed us to identify a C. fetus type A strain of reptile origin in this patient with recurrent bacteremia. To our knowledge, this is the first confirmed human infection with a C. fetus strain with markers of reptilian origin.
of reptile origin. How the patient acquired the organism is unknown. Neither the patient nor his relatives and friends kept any pet reptiles, and he denied consuming unpasteurized dairy products, which have been linked to Campylobacter fetus infection (13). Although he did not consume any reptile products near the time of his illness, he had eaten turtle soup during the prior year, and his family occasionally prepared turtle soup. Whether the characteristics we described are specific for reptile isolates or reflect a broader, as yet undefined, environmental niche is unknown. However, the deep branching observed between the mammalian and reptile isolates (16) is consistent with an ancient dichotomy. Other large-scale studies of the Campylobacter fetus sap locus are consistent with the role of Campylobacter fetus as a reservoir for Campylobacter fetus subspecies fetus infection. The occurrence of bacteremia in a debilitated host is consistent with the role of Campylobacter fetus as an opportunistic pathogen. It is possible that prophylaxis with trimethoprim-sulfamethoxazole may have predisposed to infection in this patient, since these antimicrobial agents have been used to suppress the normal flora, but most Campylobacter strains are resistant to trimethoprim (2). How often Campylobacter strains of reptile or related origin actually cause human disease remains to be determined. The fastidious nature of Campylobacter fetus and suboptimal detection in commonly used blood culture systems (20) suggest that its occurrence is underrecognized.

Nucleotide sequence accession numbers. The 16S rRNA and partial sapD sequences of the isolate have been deposited in the GenBank sequence database under accession numbers AY621303 and AY621304, respectively.

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