First Report of Treatment of *Anaerobiospirillum succiniciproducens* Bloodstream Infection with Levofloxacin[▽]

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The full extent of the clinical spectrum and optimal therapy of *Anaerobiospirillum succiniciproducens* infections remains to be determined. We describe the first case of bloodstream infection (BSI) due to *A. succiniciproducens* in an asymptomatic elderly male with poor dentition that was treated with levofloxacin.

CASE REPORT

An 81-year-old male presented to our institution for evaluation and treatment of worsening depression. His past medical history was significant for squamous cell carcinoma of the right cheek, for which he had undergone surgery and radiation therapy 15 years ago. He had a nonproductive cough for 3 months, but he denied fever, chills, nausea, vomiting, abdominal pain, or diarrhea. He had not seen a dentist for many years, and he denied any exposure to any pets, including cats or dogs. Physical examination revealed normal temperature, poor dentition, and rales at the right lung base but was otherwise unremarkable. His white blood cell count was 11,500 cells/mm³ with 82% polymorphonuclear cells, and other laboratory data were normal. A chest X ray revealed an infiltrate at the right lung base, and the patient was started on oral levofloxacin, 500 mg daily for 10 days, for empirical treatment of community-acquired pneumonia. Blood cultures (the anaerobic bottles from 2 sets of blood cultures) obtained on admission due to his pneumonia subsequently grew a spiral Gram-negative rod after 4 days. This was ultimately identified as *Anaerobiospirillum succiniciproducens*.

Bacterial growth was detected in two anaerobic bottles on day 4 of incubation using the BacT/Alert (bioMérieux, Marcy l'Etoile, France). Large Gram-negative spiral-shaped bacteria, measuring on average 6 μ m in length by 0.7 μ m in width, were detected on Gram stain (Fig. 1), and corkscrew motility was noted on wet mount. The organism was subcultured to blood agar, chocolate blood agar, and thioglycolate broth; incubated at 35°C in 5% CO₂ and brucella blood agar; and incubated at 35°C under anaerobic conditions. To rule out *Campylobacter* species, the organism was also subcultured to brucella blood agar and incubated at 35°C and 42°C in a microaerophilic environment. Anaerobic growth on brucella blood agar revealed translucent, nonhemolytic colonies; no growth was observed on

blood agar or brucella blood agar in a microaerophilic atmosphere. The isolate was oxidase, catalase, and nitrate negative. RapID ANA II (Remel, Lenexa, KS) revealed positive α -glucosidase and *N*-acetylglucosaminidase reactions but was unable to successfully identify the organism. We further confirmed the identification of *Anaerobiospirillum* spp. by investigating the specific flagellar arrangement using a transmission electron microscope. Spiral bacteria with bipolar multitrichous flagella were evident in all grid fields examined (Fig. 2).

Molecular identification of the bacterium was obtained by sequencing of the 16S rRNA gene. Whole-genomic DNA was extracted from a single colony of the bacterium using the NucleiSENS easyMag system (bioMérieux, Durham, NC). The 5' 527 bases of the 16S rRNA gene were amplified using the MicroSeq 500 PCR master (Applied Biosystems, Foster City, CA), and amplicons were treated with the ExoSAP-IT reagent (USB, Cleveland, OH). Forward and reverse sequencing reactions were performed for each amplicon using the MicroSeq 500 sequencing mix, followed by purification using a DyEx 2.0 spin column (Qiagen, Valencia, CA). Sequencing analysis was performed on an ABI 3130x genetic analyzer. Sequence data were analyzed using the SmartGene Integrated Database Network System (IDNS; SmartGene, Raleigh, NC) and revealed a 98.9% match to the reference organism, *Anaerobiospirillum succiniciproducens*. To confirm the identity of the organism, the full 16S rRNA gene was sequenced as described elsewhere (1). Briefly, two subregions of the 16S rRNA gene were amplified by using two pairs of primers. The two subregions were defined as follows: region A was an 899-bp sequence between primers 8UA (5'-AGAGTTTGA TCCTGGCTCAG-3') and 907B (5'-CCGTC AATTCMTT AGTTT-3') and region B was a 711-bp sequence between primers 774A (5'-GTAGTCCACGCTGTAAACGATG-3') and 1485B (5'-TACGGTTACCTTGTTACGAC-3'). These PCR products were then sequenced directly by an ABI Big-Dye Terminator v3.1 cycle sequencing kit on an ABI 3130 sequencer (Applied Biosystems, Foster City, CA). The sequencing data were analyzed by assembly of the reverse and forward sequences of the two fragments into a consensus sequence and by comparison of the full 16S rRNA gene with

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FIG. 1. Gram stain of a pure culture of *Anaerobiospirillum succiniciproducens*, isolated from the patient's blood.

GenBank sequences by the Basic Local Alignment Search Tool (BLAST). This analysis revealed a 99.25% match to the full 1,424 bases described for the 16S rRNA gene of *A. succiniciproducens* strain 6626 (GenBank sequence EU863654.1).

Antimicrobial susceptibility of the *A. succiniciproducens* isolate was determined by Etest (AB Biodisk, Solna, Sweden) using brucella blood agar plates. MICs were read after 24 h of anaerobic incubation and interpreted using CLSI criteria for anaerobic organisms. The *A. succiniciproducens* isolate in our case was susceptible to the antibiotics meropenem (MIC, ≤ 0.25 $\mu\text{g/ml}$), metronidazole (MIC, 8 $\mu\text{g/ml}$), penicillin (MIC, 0.5 $\mu\text{g/ml}$), and piperacillin-tazobactam (MIC, ≤ 8 $\mu\text{g/ml}$) and resistant to clindamycin (MIC, >16 $\mu\text{g/ml}$). Other antibiotics tested were levofloxacin (MIC, 0.5 $\mu\text{g/ml}$), tigecycline (MIC, ≤ 0.016 $\mu\text{g/ml}$), and daptomycin (MIC, >256 $\mu\text{g/ml}$).

Follow-up blood cultures after treatment with levofloxacin were negative. Computed tomography of the abdomen and pelvis was unremarkable. Dental evaluation revealed a fistula on the lingual side of one of his teeth, and this was felt to be the potential source of bacteremia. He was started on a complementary course of oral penicillin VK, 500 mg four times daily, until his tooth was extracted, and he finished a course of 14 days. He remained afebrile and asymptomatic and was discharged in stable condition.

Discussion. *Anaerobiospirillum* spp. are a group of spiral, motile, Gram-negative, anaerobic rods that have been isolated from the feces of mammals, including cats, dogs, and humans

FIG. 2. Transmission electron micrographs of phosphotungstic acid-stained *A. succiniciproducens*. Two separate grid fields illustrating the typical appearance of spiral cells and bipolar tufts of flagella. Magnification, $\times 5,000$ (left panel), $\times 10,000$ (right panel).

(1, 9). There are two known *Anaerobiospirillum* species that infect humans: *A. succiniciproducens*, which causes both bloodstream infections (BSI) (3, 5, 7, 10, 11, 15–17, 20–22) and diarrhea (9), and *A. thomasi*, which has been implicated only as a cause of diarrhea (8). Postinfection sequelae, notably arthritis, can also occur in some patients with *A. succiniciproducens* infection (6, 10, 19). Previous reports have suggested that the primary portal of entry in patients with BSI caused by this microorganism appears to be the gastrointestinal tract and that gastrointestinal symptoms are the most common accompanying symptoms of BSI (9, 11). Our patient most likely had a coincidental respiratory tract infection, possibly due to aspiration, and *A. succiniciproducens* bacteremia. Although a clinical diagnosis of lower respiratory tract infection was made, the possibility that the organism was simply a contaminant is unlikely, because it grew from two separate blood culture sets. The source of the bacteremia was thought to be his oral fistula. The patient had no known contact with dogs and cats. Although *A. succiniciproducens* has not been isolated from the mouth flora of humans, it was most likely part of the oral flora in our patient and his poor dentition and oral fistula precipitated this bacteremia.

Most patients with *A. succiniciproducens* BSI have underlying disorders such as alcoholism, malignancy, atherosclerosis, surgery, diabetes mellitus, and poor dentition (11). The patient whom we report had a history of malignancy, surgery, and a dental fistula, which may represent risk factors for bacteremia with *A. succiniciproducens*. All these factors, including 2 previous surgeries in the buccal mucosa, previous radiation therapy, and the presence of a fistula, may be related to a breakdown in the oral mucosa that could have precipitated bacteremia.

Differentiation from *Campylobacter* spp. is very important from epidemiologic and treatment perspectives. *Anaerobiospirillum* spp. may be mistakenly identified as *Campylobacter* spp. due to their similar shapes on Gram staining and positive motility. *Anaerobiospirillum* spp. are oxidase and catalase negative, demonstrate corkscrew-like motility, and have bipolar tufts of flagella whereas *Campylobacter* spp. are oxidase and catalase positive, display darting motility, and have a single flagellum on one or both poles (11, 21). The lower contrast of cytoplasm in the flagellar base and the tips of *A. succiniciproducens* cells, the electron-dense ring situated underneath the flagellated pole in close connection with the outer sheath, and the existence of the fibrillar structures, which are oriented along the longitudinal axis and stabilize the corkscrew shape, seem to be unique and have not been described in other bacterial species (21) (also Fig. 2).

Commercial kits such as Rapid ID 32A (bioMérieux) and API-ZYM (bioMérieux) are also being used for definitive identification of this organism. Useful biochemical markers for *Anaerobiospirillum succiniciproducens* include negative results for catalase, oxidase, indole, and nitrate reduction (20). Additional tests using API-ZYM (bioMérieux) will typically reveal positive reactions for leucine arylamidase, phosphohydrolase, glycosidase, *N*-acetylglucosaminidase, and β -galactosidase and a negative reaction with α -galactosidase. Carbohydrate fermentation is positive for fructose, glucose, maltose, sucrose, lactose, and raffinose (10, 14). According to previous reports, *A. succiniciproducens* and *A. thomasi* can be differentiated by

biochemical tests with carbohydrate fermentations and by the enzyme profiles obtained with API-ZYM strips, notably the absence of β -galactosidase and α -glucosidase activities in *A. thomasi* (8).

Determination of the 16S rRNA gene by PCR amplification and sequencing, however, is the most accurate method for identification and classification of *Anaerobiospirillum* spp. (20). However, the identity of *A. succiniciproducens* has been confirmed with a 16S rRNA sequencing test in only 6 cases (2, 12, 18, 20).

Because of the infrequency of reported cases of BSI due to this organism, the optimal antimicrobial treatment for *A. succiniciproducens* is undetermined. In most studies susceptibility data were not reported. In the few studies in which susceptibility testing results were reported, no detailed data on susceptibility for all classes of antibiotics were reported. Disk diffusion, microdilution, and Etest antimicrobial susceptibility testing on blood culture isolates have been reported in seven (3, 10, 15, 16, 19, 20, 22), three (11, 13, 14), and one (3) study, respectively. In addition *A. succiniciproducens* is often resistant to many antibiotics normally prescribed for other anaerobic infections, such as clindamycin and metronidazole (11, 14, 20, 22). This organism is reported to be susceptible to amoxicillin-clavulanic acid, cephalosporins, and chloramphenicol and resistant to vancomycin (3, 6, 11, 14). Susceptibilities to penicillin G, ampicillin, erythromycin, clindamycin, and metronidazole are variable (20). To our knowledge, this is the first report of susceptibility testing of *A. succiniciproducens* against certain antibiotics such as levofloxacin, tigecycline, and daptomycin. Although the MIC value was comparable to the MICs of other susceptible microorganisms, there are no standard interpretative criteria of these antibiotics for *A. succiniciproducens*.

Although most of the patients with *A. succiniciproducens* bloodstream infections had received antimicrobial therapy, its effect on clinical outcome is unknown: several untreated patients recovered, while several treated patients died (11). In all of these cases treatment was empirical and *A. succiniciproducens* was identified only later during hospitalization, as in our case. Duration of antimicrobial therapy was reported in only 5 cases and varied between 7 and 14 days (10, 16, 18, 20, 22). Thus, further studies are needed to define the role of antimicrobial therapy in BSI with *A. succiniciproducens*. Our patient was completely asymptomatic and afebrile, and his BSI responded to treatment with levofloxacin that was given for treatment of pneumonia. Given the reported mortality associated with *A. succiniciproducens* infection, we treated the patient with an extra course of oral penicillin for 2 additional weeks until he had his tooth extracted.

Although only 41.7% of the reported isolates with available susceptibility data were susceptible to penicillin (3, 10, 11, 13, 14, 15, 16, 19, 20, 22), *Anaerobiospirillum* infections with elevated MICs (intermediate susceptibility) may be treatable, as high levels of penicillin are achievable in blood.

Although there are no documented breakpoints for fluoroquinolones for anaerobic organisms, the MICs of 7 isolates were in the susceptible range for aerobic bacteria and *Anaerobiospirillum* was reported to be susceptible to quinolones *in vitro* (4, 10, 20). In one study 3 isolates of *Anaerobiospirillum succiniciproducens* were reported as susceptible to gemifloxa-

cin and trovafloxacin (4), whereas 4 isolates of *Anaerobiospirillum succiniciproducens* have been described to be susceptible to ciprofloxacin (10, 20). Fluoroquinolones can be useful antimicrobial agents in these cases, especially in the setting of allergy to penicillin and potential resistance of this anaerobe to clindamycin and metronidazole, which are normally common therapeutic options for anaerobic infections. It is also possible to treat these patients with oral fluoroquinolones, especially if they are afebrile and asymptomatic. Clinicians should be aware of therapeutic options for treatment of *A. succiniciproducens* infection, as this organism has been associated with significant mortality in certain cases.

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