CASE IN POINT

Bacteroides Fragilis Vertebral Osteomyelitis and Discitis: "Back" to Susceptibility Testing

LCDR John C. Chin, MD, MC, USN; CDR Tyler E. Warkentien, MD, MC, USN; Brendan W. Corey; Erik C. Snesrud; and CAPT Karl C. Kronmann, MD, MC, USN

Genetic testing of anaerobic isolates can be important for proper antimicrobial stewardship to identify the appropriate narrow-spectrum treatment for a polymicrobial infection.

John Chin is an Internal Medicine Physician; **Tyler Warkentien** and **Karl Kronmann** are Infectious Disease Physicians; all at Naval Medical Center Portsmouth in Virginia. **Brendan Corey** and **Erik Snesrud** are Researchers in the Multidrug-Resistant Organism Repository and Surveillance Network at Walter Reed Army Institute of Research in Silver Spring, Maryland.
Correspondence: John Chin (chinjoh@gmail.com)

Acute pyogenic vertebral osteomyelitis is often due to hematogenous spread of aerobic bacteria.[1-4] Conversely, only 0.5% of anaerobic bacteremias lead to osteomyelitis.[5] Anaerobic osteomyelitis typically results from the contiguous spread of polymicrobial infections through breaks in the gut mucosal barrier and involves the vertebral bodies in only 2% to 5% of cases.[5,6] Although *Bacteroides fragilis* (*B fragilis*) is the most common anaerobic pathogen cultivated from blood, accounting for about half of all anaerobic blood isolates, it seldom leads to osteomyelitis.[1,2,7-11] We report an uncommon case of *B fragilis* bacteremia and vertebral osteomyelitis confounded by uncertainties in anaerobic identification and susceptibilities.

CASE PRESENTATION

A healthy-appearing male aged 55 years presented to the Naval Medical Center Portsmouth (NMCP) with subacute low back pain and fevers of 103 °F for > 3 weeks. While traveling 4 weeks prior, he completed a course of oseltamivir for influenza B infection; afterward, he was diagnosed with community-acquired pneumonia and treated with a dose of ceftriaxone and a 7-day course of doxycycline. The patient presented to the same facility a week later for low back pain and nonresolving respiratory symptoms, and his therapy was changed to azithromycin, cefuroxime, prednisone, and inhalers. Additionally, after being treated for influenza, he developed constipation and hematochezia for which he did not seek care. The hematochezia was similar to a previous episode from an anal fissure 1 year prior that resolved with stool softeners. When he was finally seen at NMCP after 3 weeks of worsening back pain and fevers, lumbosacral magnetic resonance imaging (MRI) demonstrated vertebral osteomyelitis and discitis at L4-L5 and admitted to the hospital (Figure 1).

After a fluoroscopy-guided biopsy of the L4 vertebral body on hospital day 1, the patient was started on cefepime and vancomycin. The biopsy sample was inoculated onto solid media (blood agar, chocolate agar, and MacConkey agar) and incubated at 36 °C for 24 hours in a 5% CO_2 atmosphere, as well as onto Shaedler agar with vitamin K and chopped meat glucose broth and incubated at 36 °C for 48 hours under anaerobic conditions. Metronidazole was added and vancomycin discontinued after 2 anaerobic blood culture vials obtained on hospital day 1, incubated in a Becton Dickinson BACTEC FX automated system, which demonstrated Gram-negative bacilli after 48 hours. The blood culture isolates demonstrated a > 99% probability of being identified as ß-lactamase positive *Prevotella loescheii* using Thermo Fischer Scientific RapID ANA II biochemical testing. Nitrocefinase discs were used to detect ß-lactamase activity.

The biopsy demonstrated nongranulomatous focal areas of necrotic bone and neutrophilia in a hematopoietic background consistent with acute osteomyelitis (Figure 2); on hospital day 4, ß-lactamase positive *B fragilis* grew from the bone culture. Additionally, 1 anaerobic vial from a surveillance blood culture set that was obtained on hospital day 3 grew ß-lactamase positive *B fragilis* using the same identification methods. With these results he was thought to have a polymicrobial infection (*B fragilis* and *Prevotella loescheii* [*P loescheii*])

from a suspected bowel source based on his hematochezia and history of anal fissure. No aerobic, Gram-negative enterobacteriaceae were isolated, but he had previously been on cefuroxime, which has potential activity against these organisms, for ≥ 2 weeks prior to hospitalization and cultures. He was discharged on moxifloxacin and metronidazole pending final culture results, including requested anaerobic susceptibility testing.

At 1-week follow-up, both aerobic and anaerobic vials from surveillance blood cultures remained negative for any microbes, so antibiotics were deescalated to moxifloxacin monotherapy. However, after 3 days the patient was readmitted for increasing C-reactive protein (CRP) levels and intractable back pain with worsening bilateral radiculopathy. A repeat MRI demonstrated interval disease progression with near obliteration of the L4-L5 disc space and hyperenhancement of the prevertebral soft tissues and adjacent psoas musculature without focal rim-enhancing fluid collection (Figure 3). After repeat L4 biopsy, metronidazole was restarted and ertapenem added for enterobacteriaceae coverage, given the known *B fragilis* and potential suppression from previous cephalosporin therapy; moxifloxacin was discontinued. L4 biopsy cultures showed no growth, and CRP levels trended down from 154.2 mg/L (start of first admission) to 42.4 mg/L (start of second admission) to 14.9 mg/L (day of discharge) (reference range, 5-9.9 mg/L). He was discharged on ertapenem and metronidazole. He completed a 6-week course without further complication.

During antibiotic therapy he had an unremarkable colonoscopy, CRP normalized to 2.6 mg/L (reference range, 0-4.9 mg/L), and he underwent successful L4-L5 transforaminal lumbar interbody fusion 2 weeks after finishing antibiotics.

We retroactively sent both *P loescheii* isolates and the 1 *B fragilis* isolate that grew from the surveillance blood culture to the Multidrug-resistant Organism Repository and Surveillance Network (MRSN) at the Walter Reed Army Institute of Research for identification confirmation and susceptibility analysis. Whole genome sequencing with single nucleotide polymorphism (SNP)-based analysis revealed all isolates were 100% identical and consistent with *B fragilis* and not *P loescheii*, based on clustering around other *B fragilis* sequences found in the National Center for Biotechnology Information (NCBI) Genbank database (Figure 4). All isolates carried the antibiotic resistance genes— *cepA, sul(2), tetQ*— encoding for possible resistance to cephalosporins, sulphonamides, and tetracyclines, respectively; as well as a point mutation in the *gyrA* gene (Ser82Phe). None of the isolates carried the *nim* gene, and screening for the 3 subtypes of *B fragilis* enterotoxin gene (*bft-1, bft-2, bft-3)* was negative. Eventual susceptibility testing at the Mayo Clinic several months after the conclusion of the case indicated that the *B fragilis* isolate was

FIGURE 1 Initial Sagittal Section Lumbar Magnetic Resonance Image With Gadolinium

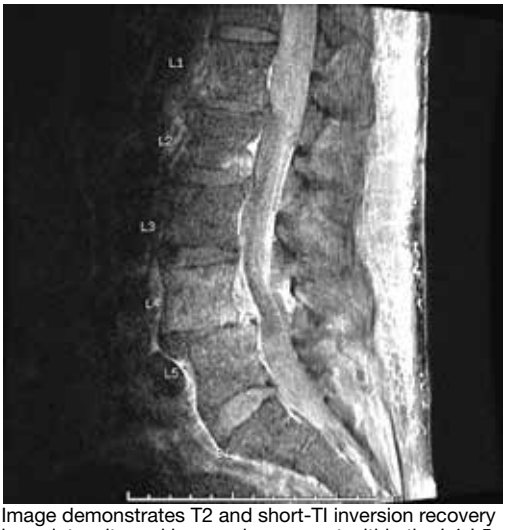


Image demonstrates T2 and short-TI inversion recovery hyperintensity and hyperenhancement within the L4-L5 vertebral bodies and marked disc space narrowing.

FIGURE 2 Necrotic Bone Demonstrating Neutrophil Clusters Consistent With Osteomyelitis

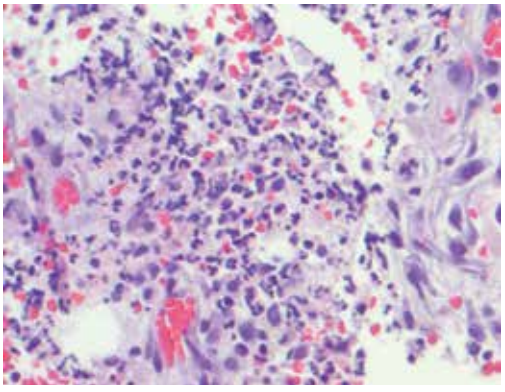


FIGURE 3 Repeat Magnetic Resonance Image

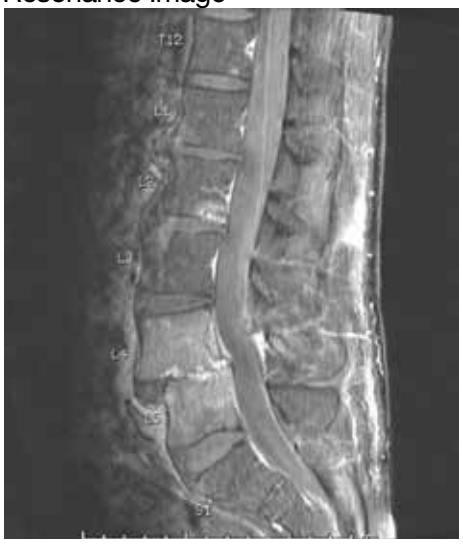
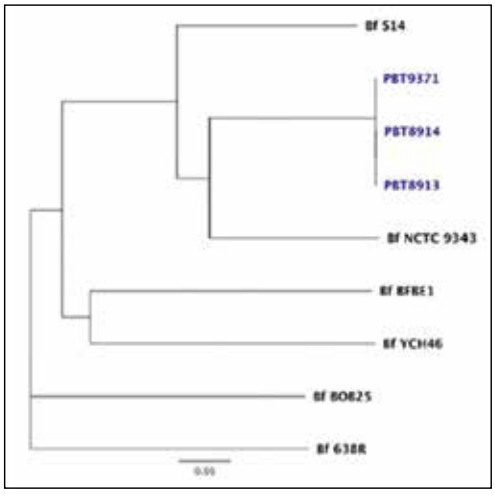


Image demonstrates worsening disease and near complete obliteration of the L4-L5 disc space.

FIGURE 4 Whole Genome Sequence Dendrogram



Genbank sequence indicates 3 identical *Bacteroides fragilis* isolates (PBT 9371, 8914, 8913) and other *Bacteroides fragilis* sequences; branch lengths are indicative of strain relatedness.

sensitive to piperacillin-tazobactam, ertapenem, clindamycin, and metronidazole; however, testing was not performed against moxifloxacin.

DISCUSSION

In the era of growing antibiotic resistance patterns, antimicrobial stewardship programs recommend interventions to improve antimicrobial use through targeted narrow-spectrum antibiotics.[12] The Clinical and Laboratory Standards Institute (CLSI) maintains guidelines on the major indications for anaerobic antimicrobial susceptibility testing (AST) to help direct narrow-targeted antimicrobial therapy. However, in a 2008 practice survey Goldstein and colleagues reported that less than half of US hospitals performed anaerobic AST, and only 21% of these facilities did it in-house, while the remainder sent out their isolates for testing.[11-14] The CLSI major indications for AST include situations in which the selection of agents is important because of the (1) known resistance of a particular species; (2) confirmation of appropriate therapy for severe infections or for those that may require long-term therapy; (3) persistence of infection despite adequate treatment with an appropriate therapeutic regimen; and (4) difficulty in making empirical decisions based on precedent.[14] Additionally, isolates from brain abscess, endocarditis, osteomyelitis, joint infection, infection of prosthetic devices or vascular grafts, bacteremia, and normally sterile body sites (unless contamination suspected) should be tested.[14]

Because of the lack of anaerobic AST, health care providers must base empiric treatment on reported sensitivities from the medical literature. Empiric selection of antimicrobials for anaerobic infections is made even more challenging by the increased rates of resistance reported in the literature, leading to recommendations to increase susceptibility testing to guide therapy.[13,15,16] Empiric therapy of deep-seated anaerobic infections may lead to use of inactive agents or overly broad-spectrum antibiotics. Current antimicrobial stewardship initiatives recognize the importance of narrow-spectrum antibiotics to minimize risk of adverse events and selective pressure for antimicrobial resistance.

Although we attempted to confirm the identification of the anaerobic isolates via commercially available methods, it was not until we performed genetic testing that we were able verify the isolates as *B fragilis*. Furthermore, earlier susceptibility testing would have allowed for more narrow-targeted antimicrobial therapy and could have potentially prevented our patient's readmission and use of ertapenem, despite its > 98% susceptibility rates against *B fragilis*.[13,17]

All of the *B fragilis* isolates carried the *cepA* gene, which is a cephalosporinase that encodes for resistance to cephalosporins and aminopenicillins but not to ß-lactam ß-lactamase inhibitor combinations.[13] Although not a substitution for susceptibility analysis, genetic testing showed that all of the isolates carried a nonsynonymous mutation from serine to a phenylalanine at amino acid position 82 (S82F) in the *gyrA* gene. The S82F mutation has been implicated in fluoroquinolone resistance, via inhibition of substrate–target recognition and binding between fluoroquinolones and the

target topoisomerase protein,[18] and may potentially explain why our patient clinically worsened while on moxifloxacin monotherapy. Although moxifloxacin susceptibility was not performed, susceptibility rates remain highly variable, ranging from 50% to 70% for *B fragilis*.[13,15,16]

It is important to note that the metronidazole the patient received during his first hospital admission could have sterilized the vertebral body without completely eradicating the microbe; thus could explain his clinical worsening while on moxifloxacin monotherapy despite no growth from the repeat biopsy culture. Our rationale for initially continuing moxifloxacin was based on its excellent bioavailability and bone penetration properties. Additionally, of the fluoroquinolones it has the most reliable anaerobic activity and is the only one recommended as monotherapy for complicated intraabdominal infections.[19] However, guidelines recommend avoiding its use in patients who have received a fluoroquinolone in the past 90 days or at institutions with high rates of resistance. At our institution *Escherichia coli* has a > 90% susceptibility rate to fluoroquinolones. Given this rate and our concern that the patient had a polymicrobial infection, we felt that moxifloxacin would provide appropriate anaerobic and aerobic coverage, especially since he had no previous fluoroquinolone exposure.

Additionally, none of the isolates carried the *nim* or *bft* toxin genes. Although the *nim* gene is associated with metronidazole resistance, its presence does not invariably result in resistant strains of *B fragilis*; in fact, metronidazole resistance is relatively uncommon, with the majority of *B fragilis* showing < 1% resistance, based on CLSI breakpoints (≥ 32 mg/L).[13,20,21] However, one recent epidemiologic study on anaerobic wound isolates from Iraq and Afghanistan casualties found that 12% (2/17) of *B fragilis* isolates were resistant to metronidazole.[15] Given the improvement of the patient's symptoms while on metronidazole, it is likely that the *B fragilis* was susceptible. Nevertheless, susceptibility testing with minimum inhibitory concentrations is necessary to verify this result. Also, although enterotoxigenic strains of *B fragilis* have been associated with bloodstream infections, our patient's isolates lacked the 3 subtypes of *B fragilis* enterotoxin gene.[22]

CONCLUSIONS

We report a case of *B fragilis* bacteremia and vertebral osteomyelitis complicated by challenges in anaerobic identification and sensitivities that led to brief use of a possibly inactive antimicrobial and the subsequent use of carbapenem therapy, which may have been avoided if susceptibility testing were more readily available. This case led to changes in our hospital's processing of anaerobic isolates to include susceptibility testing on request.

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Author disclosures

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