Bordetella holmesii, an Emerging Cause of Septic Arthritis

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***Bordetella holmesii* is a well-described pathogen in asplenic and immunocompromised patients. Here we report the first two published cases of septic arthritis caused by *B. holmesii* documented in apparently immunocompetent patients and unaccompanied by bacteremia.**

CASE REPORTS

Case 1. A 54-year-old woman presented to the hospital with a suspected right prosthetic knee infection. She had a past medical history significant for hypertension, dyslipidemia, and a bilateral knee replacement in 2008 secondary to osteoarthritis, but no history of frequent or unusual infections. She had experienced chronic pain in her right knee since the initial surgery; because of this, she underwent arthroscopy in July 2010 with a debridement of the meniscus, but the pain did not improve. The pain remained stable until November 2010 when she experienced an acute worsening of her right knee pain associated with swelling, an inability to bear weight, and a low-grade fever. There was no history of corticosteroid injections into the joint for symptom control.

On initial examination, the patient had a temperature of 37.9°C and was hemodynamically stable. Her right knee was swollen, tender, and very warm, with a limited range of motion. The remainder of her physical examination was noncontributory.

Blood work showed a white blood cell (WBC) count of 9.1×10^9 /liter, C-reactive protein (CRP) of 327 mg/liter, and erythrocyte sedimentation rate (ESR) of 90 mm/h. The right knee was aspirated under local anesthesia, and the cell count was $26,060 \times 10^6$ /liter (94% neutrophils); Gram stain of the aspirate did not show organisms, and bacterial culture was negative after 48 h. There was no growth of any organism in blood culture.

The patient was started empirically on intravenous (i.v.) cefazolin and was taken to surgery for a first stage patellofemoral revision with prosthesis removal and replacement by a vancomycin and tobramycin cement spacer.

The specimen from the initial knee aspirate was first reported as growing a Gram-negative bacillus after 5 days of culture, after the patient had already undergone surgery. There were four small, gray colonies growing on the blood agar plate incubated aerobically in 5% CO₂ at 35°C. The MacConkey, chocolate agar, and anaerobic plates showed no growth. Gram stain of the culture revealed small Gram-negative bacilli and coccobacilli with much variation in size. Oxidase, urease, and catalase tests were negative. The organism was nonmotile in wet prep and semisolid media incubated at 35°C. There was no growth on a triple sugar iron (TSI) slant. A RapID NH panel (Innovative Diagnostics, Atlanta, GA) was set up but was nonreactive. The patient was switched from cefazolin to ceftriaxone pending identification of the Gram-negative bacterium.

The isolate was sent to a reference laboratory but could not be definitively identified by biochemical analysis. Sequencing of a 635-nucleotide region of the 16S rRNA gene was undertaken; this alignment was compared to the NCBI (GenBank plus EMBL plus DDBJ plus PDB sequences) database using BLASTN 2.2.25+ and was found to have 99% homology with *Bordetella holmesii*. Part of the sequence also was a 99% match with *Bordetella bronchiseptica*, but the isolate was urease negative and nonmotile, which ruled out that possibility. Gas chromatography using fatty acid methyl ester analysis (GC-FAME) was performed in selective ion monitoring (SIM) mode and compared to the Sherlock Midi database (version 6.0B) to identify the organism as *B. holmesii*, and this identification was confirmed using matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS). The isolate exhibited the following susceptibilities: ceftazidime MIC < 8 µg/ml, ciprofloxacin MIC < 1 µg/ml, gentamicin MIC < 4 µg/ml, and trimethoprim-sulfamethoxazole MIC < 2/38 µg/ml. Consequently, it was reported as being “susceptible” to these antimicrobial agents based on Clinical and Laboratory Standards Institute (CLSI) breakpoints for non-*Enterobacteriaceae* and nonfermenters other than *Pseudomonas aeruginosa*, *Acinetobacter* spp., *Burkholderia* spp., or *Stenotrophomonas maltophilia* (1) though the applicability of these breakpoints for a fastidious organism is questionable. Subsequent cultures from operative tissue specimens also grew the same organism. The patient was started on treatment with ciprofloxacin. The patient was given 750 mg of ciprofloxacin orally (p.o.) twice a day (BID) for 2 months and clinically improved. Six weeks after discontinuation of antibiotics, the patient had the prosthesis replaced; all cultures taken during that operation were negative.

Case 2. A 15-year-old previously healthy boy presented with a fever and a swollen knee. Three days prior to being admitted to the hospital, he had experienced blunt trauma to his right knee. The pain was short-lived, but 2 days later, he developed a fever and

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recurrence of the pain. There was no preceding history of respiratory symptoms.

He had no significant past medical history, including no previous invasive bacterial infections and no adverse reactions to vaccines. His medical history was normal, and he was on no regular medications.

On initial examination, his right knee was swollen, warm, and tender with limited range of motion. An X-ray of the knee was normal. His initial WBC count was 10.0×10^9 /liter, his ESR was 4 mm/h, and his CRP level was 75.7 mg/liter. Blood cultures were sent and returned negative after 5 days. A knee aspirate was performed; the synovial fluid showed no organisms on Gram stain and had a WBC count of $54\,000 \times 10^6$ /liter. Incision and drainage were performed on the day of admission, and he was subsequently started on cefazolin. He was given 1 g of cefazolin i.v. every 8 h (q8h). The patient defervesced 1 day after his operative procedure, and his knee progressively improved. Blood cultures drawn prior to antibiotic therapy were negative. After 3 days, synovial fluid culture revealed a small oxidase-negative Gram-negative bacillus growing on the chocolate agar plate; there was no growth on MacConkey agar. Due to the isolate's poor growth, identification was not possible using the Vitek 2 system (bioMérieux, St. Laurent, Quebec). The patient's antimicrobial therapy was changed to ceftriaxone. His CRP dropped to 7.3 mg/liter 5 days after admission and was normal from that point forward; his ESR never increased.

He had normal levels of immunoglobulins (IgG, IgM, and IgA), normal tetanus and diphtheria titers, and a normal CH50 (the CH50 measures the total hemolytic activity of a test sample and is the reciprocal of the dilution of serum complement needed to lyse 50% of a standardized suspension of sheep erythrocytes coated with antierythrocyte antibody). An ultrasound confirmed he had a spleen. Serial complete blood counts showed normal levels of all leukocytes. HIV testing was not done.

After 2 weeks of ceftriaxone therapy, his knee had improved somewhat, but a small effusion remained. At that point, the bacterial isolate was identified as *B. holmesii* by GC-FAME as described above. Sequencing of a 717-nucleotide region of the 16S rRNA gene was done, compared to the NCBI database using BLASTN 2.2.25+, and found to be 99% homologous with *B. holmesii*. The isolate was found to be asaccharolytic as shown by oxidation and fermentation of sugars and biochemically inert, consistent with *B. holmesii*. Susceptibilities could not be determined, due to the poor growth of the isolate. There were concerns about his slow recovery, and he was experiencing facial flushing with the ceftriaxone, so the patient was switched to oral levofloxacin (750 mg p.o. daily), based on reports in the literature documenting low fluoroquinolone MICs for most *B. holmesii* isolates. After 1 month of levofloxacin therapy, the swelling had resolved, though flexion around the knee was still significantly decreased because of pain. The antimicrobials were continued because of his less-than-complete response; after 7 weeks of levofloxacin, he was back to full functionality, and the range of motion around his knee had almost completely normalized.

Discussion. *B. holmesii* was first isolated in 1983 and was designated a CDC nonoxidizer group 2 (NO-2), and identified as a member of the genus *Bordetella* in 1995 (2). The genus *Bordetella*

includes the more familiar *B. pertussis*, the causative agent in whooping cough, as well as *B. parapertussis* and *B. bronchiseptica*, which also cause human respiratory tract infections.

B. holmesii is a fastidious, slow-growing, asaccharolytic, small Gram-negative bacillus with much variation in size (2, 3). It forms small colonies on sheep blood agar and is negative for oxidase, nitrate reduction, urease, and spot indole (2, 3). The organism is nonmotile in wet preparation and semisolid media incubated at 35°C. In addition to differing from *B. pertussis* by virtue of being oxidase negative, *B. holmesii* differs in that it produces a brown diffusible pigment in solid-phase media; this also differentiates it from *Acinetobacter* species (2, 3). *B. holmesii* may be an underrecognized pathogen due to its slow growth and difficult identification. There have been reports of repeated misidentifications of this organism as *Acinetobacter lwoffii* using standard automated laboratory systems; the Vitek2 automated system fails to identify *B. holmesii* because the microorganism is not included in its database (4). Cellular fatty acid profiles and 16S rRNA gene sequencing are useful for making a definitive identification (3). Matrix-assisted laser desorption ionization–time of flight mass spectrometry has also been shown to have a role in the identification of many bacteria; this technology has been used to successfully identify *B. holmesii* (5, 6).

Case reports have identified various clinical manifestations of *B. holmesii* infection. Like other members of the genus *Bordetella*, *B. holmesii* had been associated with respiratory illness, albeit very infrequently. This organism was isolated from 33 of 10,935 nasopharyngeal specimens taken from individuals with pertussis-like symptoms in Massachusetts between 1995 and 1998 (7) and in 12 of 10,254 nasopharyngeal samples submitted to laboratories in Ontario province in Canada in 2007 to 2008 (8). However, recent data suggest that this pathogen can be found more commonly in the context of respiratory disease, and especially in adolescents (9). During the 2010 pertussis outbreak in Franklin County, Ohio, there were 918 reported cases of pertussis. In a convenience sample of 298 swabs tested with multiplex PCR, 164 were positive for *Bordetella* spp., *B. holmesii* was detected in 48 (29%), *B. pertussis* was found in 112 (68%), and both pathogens were found in 4 specimens (2%). The majority of *B. holmesii* isolates (63%) were detected in youth from 11 to 18 years old, an important distinguishing feature from *B. pertussis*, though clinical features of both infections were similar.

Invasive *B. holmesii* infection has most often been described in the context of bacteremia in asplenic and other immunocompromised patients, with septic arthritis having been rarely reported. Shepard et al. reviewed 26 patients with *B. holmesii* bacteremia (10). Eleven patients (42%) were given a primary discharge diagnosis of bacteremia. There was one patient who had a primary diagnosis of septic arthritis, and the remaining patients were diagnosed with viral syndrome, endocarditis, pneumonia, sickle cell crisis, pyelonephritis, and cellulitis. In this review, 22 of 26 patients with *B. holmesii* bacteremia were asplenic (anatomically or functionally), 1 had AIDS, 1 was a renal transplant recipient, and one was an elderly patient on long-term steroids for rheumatoid arthritis; the sole patient without evident immunodeficiency was an infant (10). A recent report described four adolescents lacking normal splenic function who presented with mild febrile illness and had *B. holmesii* bacteremia (4); there has also been a description of septic arthritis caused by *B. holmesii* in an adolescent with chronic hemolytic anemia who was asplenic and had no concom-

itant bacteremia (11). A number of other very recent reports document pericardial and endocardial infection with *B. holmesii* in immunocompromised adults (5, 12, 13).

B. holmesii invasive infection has been described in two immunocompetent people over the age of 1 year in the literature, both of whom were adolescents (6, 14). One adolescent presented after 6 months of progressive systemic and respiratory symptoms and was found to have interstitial and lobar pneumonia with progression to pulmonary fibrosis, mediastinal collections, pericarditis, hepatosplenomegaly, pancreatitis, lymphopenia, thrombocytopenia, and coagulopathy (14). The other was a 12-year-old with anorexia nervosa (body mass index [BMI] of 16.9 kg/m² at admission) who presented with meningismus, a polymorphonuclear pleocytosis, and who had *B. holmesii* identified from cerebrospinal fluid by MALDI-TOF MS, 16S rRNA sequence analysis, and cellular fatty acid analysis (6).

Various antimicrobial therapies have been employed in the treatment of *B. holmesii* infection (10). Unfortunately, there are no established interpretive breakpoints for this organism (10). The isolates identified by Shepard et al. demonstrated higher MICs to beta-lactams, including expanded-spectrum cephalosporins, suggesting that these drugs are inferior for the treatment of *B. holmesii* infection (10). Our experience was certainly consistent with this, as both of our patients improved slowly when treated with ceftriaxone. In contrast, *B. holmesii* isolates that appeared sensitive to all antimicrobials tested were described in a more recent case series of four patients (4). It has been demonstrated that MICs of erythromycin in *B. holmesii* isolates are generally higher than those in *B. pertussis* isolates, suggesting that first-line antimicrobials against *B. pertussis* are less useful against this pathogen (15). It may be that fluoroquinolones and carbapenems are optimal therapy for *B. holmesii*, given the uniformly low MICs reported for these antimicrobials in the literature.

In conclusion, we have described the first two cases of septic arthritis caused by *B. holmesii* in patients without obvious immune dysfunction. We did not fully rule out immune deficiency in the two patients described in this report; however, the younger patient was not asplenic, had normal leukocyte counts, and had evidence of normal humoral immune function, while the older patient had not had any other significant infections (opportunistic or otherwise) in her 54 years of life. Evidence is accumulating to suggest that *B. holmesii* may cause more clinical disease than currently recognized, especially in adolescents. It remains possible that this relatively fastidious organism accounts for a proportion of "culture-negative" infective arthritis. Further studies and a heightened awareness of the importance of *B. holmesii* are needed.

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