**ABSTRACT**

**Objectives:** To identify the gram-negative rods grown from blood cultures and a right-knee fluid aspirate from an 80-year-old caucasian man who had undergone a total right knee arthroplastic procedure 6 years ago, and to assess the genetic similarity between the 2 isolates.

**Methods:** We used 3 different approaches: biochemical testing, matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) mass spectrometry, and 16S ribosomal RNA (rRNA) gene sequencing.

**Results:** The 3 methods identified the gram-negative rods as *Pasteurella multocida*; 16S rRNA gene sequencing further identified the organisms as *P. multocida* subsp. *septica*.

**Conclusion:** A concordant identification of *P. multocida* was observed using biochemical testing, mass spectrometry, and 16S rRNA gene sequencing. Only 16S rRNA sequencing was able to determine the subspecies of *P. multocida* and to determine the genetic relatedness of the 2 isolates.

**Keywords:** bacteremia, prosthetic joint, gram-negative coccobacilli, *Pasteurella multocida*, MALDI-TOF mass spectrometry, 16S rRNA gene

*Pasteurella multocida* (*P. multocida*) is a facultative anaerobic, gram-negative, small, pleomorphic, nonflagellated coccobacillus with bipolar staining that belongs to the *Pasteurellaceae* family. *P. multocida* is divided into 3 subspecies (*multocida*, *septica*, and *gallicida*), 16 somatic serovars, and 5 capsular serogroups (A to F, excluding C) based on a capsular antigen. *P. multocida* often exists as a commensal organism in the normal microbiota of the oral, nasopharyngeal, and upper respiratory tracts of many livestock, poultry, and domestic pet species; cats and dogs have the highest carriage rates, at 70% to 90% and 20% to 50%, respectively. *P. multocida* infection in humans is primarily acquired through contact with animals, most often through bites, scratches, licks on skin abrasions, or exposure to mucous secretions from pets. In the United States, according to the 2015-2016 American Pet Products Association (APPA) National Pet Owners Survey, 65% of United States (US) households own a pet, which translates to approximately 77.8 million pet dogs and 85.8 million pet cats. With the abundance of cats and dogs in US households and the commensal nature of the bacteria, the risk of acquiring *Pasteurella* infections is increasing, particularly in patients who have predisposing factors such as prosthetic joints, malignant neoplasms, and immunosuppression.

**Case Report**

The patient was an 80-year-old Caucasian man who arrived at the emergency department (ED) of Fairview Southdale, Minneapolis, MN, via the Emergency Medical Service (EMS) for evaluation of leg weakness. In the early morning hours, while attempting to rise from bed to use the bathroom, the...
patient discovered that his legs were weak and he was unable to stand. Later that morning, the patient had a similar episode of leg weakness, prompting the EMS to bring him to the ED for evaluation. The patient complained of shortness of breath, bilateral leg weakness, and right knee pain for the past 2 weeks, intermittent fever for the past week, and decreased fluids and food intake on the day of admission. His past medical history was relevant: he had undergone a right total-knee arthroplastic procedure 6 years ago; mitral and tricuspid valve repair 10 months before admission; and atrial fibrillation, for which he was taking generic warfarin sodium (Coumadin; Bristol-Myers Squibb).

On physical examination, the patient appeared to be in moderate distress and was slightly diaphoretic. His vital signs were as follows: blood pressure, 82/72 mmHg; pulse rate, 129 beats per minute; oral temperature, 39 °C; respiratory rate, 21 breaths per minute; and oxygen saturation, 97% when breathing room air. His entire lower extremities showed intact skin, as well as palpable pulses with intact motor and sensory neurologic functioning on examination. There was a well-healed midline incision on the anterior right knee, with tear effusion and no erythema or warmth. Tenderness was noted laterally along the right knee, superiorly in the suprapatellar pouch, and medially. The range of motion of the right knee and right hip was limited, passively and actively. Examination of the left leg revealed intact neurovascular status, with full range of motion and no tenderness. The lymphatic examination results were negative.

The chemistry panel results were significant for a C-reactive protein (CRP) level of 206.0 mg per L (0.86-1.14). The international normalized ratio (INR) was 3.60 (0.86-1.14). The white blood cell count was 8.6 × 10⁹ per L (4.0-11.0 × 10⁹/L), and the hemoglobin level was 11.0 g per dL (13.3 to 17.7 g/dL), and platelet count of 143 × 10⁹ per L (150-450 × 10⁹/L). The international normalized ratio (INR) was 3.60 (0.86-1.14).

Two sets of blood culture were obtained. The patient’s chest x-ray for the patient showed clear lungs with no apparent pleural effusion. X-rays of the right knee were performed, and it was reported that right total-knee arthroplasty had been performed on the patient (Image 1). The patient was treated with piperacillin/tazobactam and vancomycin and sent to the intensive care unit (ICU) for sepsis management. The Department of Orthopedics was consulted, and right knee joint aspiration was performed the following day.

Both sets of blood cultures were flagged as having bacterial growth after 1 day of incubation. On Gram staining, we observed pleomorphic gram-negative organisms with ovoid short bacilli (coccobacilli) and short chains (Image 2A) also, small gray colonies grew on 5% sheep’s blood and chocolate agars, but no growth was observed on MacConkey agar. The isolate was referred to the matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) instrument for identification; the result was P. multocida.

The fluid aspirate from the right knee appeared purulent, and Gram staining showed moderate gram-negative coccobacilli and many polymorphonuclear leukocytes (Image 2B). The fluid was plated directly onto a blood agar plate and a chocolate agar plate, as well as into a thioglycollate broth. From the broth only, on the second day of incubation, P. multocida was also isolated. Both isolates were catalase, oxidase, indole positive, and reduced nitrate to nitrite, consistent with the identification of P. multocida.

The isolates were susceptible to ampicillin, ceftriaxone, levofloxacin, penicillin, and trimethoprim/sulfamethoxazole. In addition, pure colonies from the blood and fluid culture were processed independently for nucleic acid extraction and sequencing. The 16S rRNA gene region was amplified by polymerase chain reaction (PCR) and sequenced using an Applied Biosystems 3130 Genetic Analyzer (Life Technologies). We analyzed the forward and reverse sequences produced, using Sequencher v4.8 software (Gene Codes Corporation), and a final sequence was generated. We performed searches on the final sequences for both isolates within the GenBank database using the BLAST algorithm, and P. multocida was the best sequence match, at 100% (AY299314.1), for both isolates. The sequences were then compared against the Ribosomal Database Projects database; again, P. multocida contained the highest similarity score, at 0.986/1.000 (RDPII S000389472/ GenBank: AF225205). Both databases also showed that the isolates were most closely related to P. multocida subsp. septica. Further, to determine the similarity between both isolates, their sequences were aligned to one another using the National Center for Biotechnology Information (NCBI) nucleotide BLAST function.

This alignment showed that both sequences were a 100% match.
After further investigation, we discovered that the patient owned a Pomeranian dog that nipped at his legs periodically. However, the patient did not recall having any actual infected wounds or any major skin breakdown.

The patient received a resection of his right total-knee arthroplasty and placement of an antibiotic spacer with tobramycin. His echocardiogram result was negative for vegetations. He was treated with intravenous (IV) ceftriaxone for 6 weeks. After the completion of his antibiotic course the patient underwent a revision of his right total knee arthroplasty with no complication.

Discussion

In the case of this patient, his history of owning a pet dog was discovered only after we identified that he was septic with *P. multocida*. This turn of events suggests that many physicians are still not considering the possibility of zoonotic infections in diagnosis of patients. Cat bites and cat scratches are more commonly reported as the source of infection than dogs in *Pasteurella* prostatic joint infections (PJIs), and the knee joints are more commonly affected than the hip joints. Older age is a known risk factor for *P. multocida* PJIs; our patient was 80 years old. This case is unique because the knee exam showed only tenderness and pain with range of motion but no erythema, warmth, or obvious effusion, as one might expect with joint infection, and his X-ray results were unremarkable. The *P. multocida* bacteria were introduced possibly through small skin breaks that caused bacteremia and subsequently hematogenously seeded to the right knee, or the bacteremia present on admission was secondary to the infected right knee joint. *Pasteurella* infections that are not directly associated with bite wounds often occur due to *P. multocida* species. Commonly, affected patients have severe comorbidities and/or immunocompromise. These patients
usually have bacteremia, need ICU management, and have increased mortality.\(^3\)

Subspecies of \textit{P. multocida} clinical isolates are rarely reported in the literature. The MALDI-TOF mass spectrometry system does not identify \textit{P. multocida} to the subspecies level, and the differentiation of the 3 subspecies via biochemical testing can be challenging. In our case, the 16S rRNA gene sequencing showed a close genetic relatedness to \textit{P. multocida subsp. septica}.

The identification of \textit{P. multocida} to the subspecies level can have clinical and epidemiological implications. \textit{P. multocida subsp. multocida} and \textit{P. multocida subsp. septica} are more frequently associated with bacteremia. \textit{P. multocida subsp. multocida} has been found in dog- and cat-associated injuries, whereas \textit{P. multocida subsp. septica} most often has been isolated from wounds afflicted by cats. To our knowledge, we are the first to report a case of \textit{P. multocida} PJIs associated with bacteremia for which the identification of the 2 isolates was made by sequencing the 16S rRNA gene.

The leading organisms responsible for PJIs are \textit{Staphylococcus aureus} and coagulase-negative \textit{Staphylococcus};\(^5\) \textit{P. multocida} is still a relatively rare cause of PJIs. Review of the literature since 1975 shows approximately 33 reported cases of PJIs with \textit{P. multocida}; in each case, contact with a cat or dog through a bite, scratch, or lick was reported.\(^5,7,8\)

\textit{Pasteurella} spp. generally are susceptible to penicillin, broad-spectrum cephalosporins, azithromycin, tetracyclines, and quinolones. Resistance to erythromycin has been reported. In our case individual, both isolates were susceptible to ampicillin, ceftriaxone, levofloxacin, penicillin, and trimethoprim/sulfamethoxazole. Prolonged therapy of 6 weeks of IV antibiotics was instituted in our patient to protect his repaired mitral and aortic valves from infection, as well as to treat his knee-joint infection. At follow up the patient finished his 6 weeks of antibiotic IV therapy. The patient underwent a revision of his right total knee arthroplasty without complication and was discharged to the nursing home setting 3 days after the procedure.

The high prevalence of pets in American families increases the risk of exposure to \textit{P. multocida} and thus to possible infections. It is important for clinical microbiology laboratories to identify this organism accurately and in a timely manner. We have shown that 3 different methods were able to identify this organism accurately, but only the 16S rRNA gene sequencing was able to determine its subspecies. LM

\textbf{Image 2}

Gram staining of specimens from our patient, an 80-year-old Caucasian man. \textbf{A}, Blood culture gram-negative coccobacilli (oil, original magnification \(\times 1000\)). \textbf{B}, Right-knee fluid aspirate; arrows point to gram-negative coccobacilli (oil, original magnification \(\times 1000\)).
References