CASE REPORT

Bilateral periprosthetic joint infection caused by Salmonella enterica serotype Enteritidis, and identification of Salmonella sp using molecular techniques

Hideo Kobayashi a,b, Gerri S. Hall a, Marion J. Tuohy a, Ulf Knothe b, Gary W. Procop a, Thomas W. Bauer a,b,*

a Pathology and Laboratory Medicine Institute, The Cleveland Clinic, 9500 Euclid Avenue, Cleveland, Ohio 44195, USA
b Orthopaedic and Rheumatologic Institute, The Cleveland Clinic, Cleveland Ohio, USA

Received 29 September 2008; received in revised form 17 November 2008; accepted 15 December 2008
Corresponding Editor: William Cameron, Ottawa, Canada

Summary Salmonella septic arthritis is rare. Our objective was to identify bacterial species from joint fluid using a broad-range real-time PCR and pyrosequencing technique. We describe a case of bilateral Salmonella enterica serotype Enteritidis infection of right and left total knee arthroplasties. DNA was extracted from the joint fluid of the left knee, amplified by PCR, and the amplicons were evaluated by pyrosequencing. The patient was treated with ciprofloxacin, and the polyethylene liners were replaced in both knees. The results of pyrosequencing detected a Salmonella species. To the best of our knowledge, this is the first report describing the detection of Salmonella in joint fluid by universal PCR followed by pyrosequencing.

© 2009 Published by Elsevier Ltd on behalf of International Society for Infectious Diseases.

Introduction

Periprosthetic infection is an important complication of joint arthroplasty, and occurs in association with 0.5% to 4% of primary total knee arthroplasties. The cause is usually a Staphylococcus species (mainly Staphylococcus aureus and Staphylococcus epidermidis), which account for more than 50% of cases. Salmonellosis commonly presents as gastroenteritis, but bacteremia may occur with this disease. Septic arthritis is a rare consequence of Salmonella bacteremia, noted in <1% of cases in several large reviews of salmonellosis, but the presence of a prosthetic implant may change the likelihood of complications from an otherwise transient bacteremia.

Although microbiological culture is currently considered the gold standard for diagnosis of septic arthritis, molecular methods have recently been developed to detect bacteria. Pyrosequencing (Biotage AB, Uppsala, Sweden) technology is...
a novel method of nucleic acid sequencing-by-synthesis that is rapid and reliable. This technique has been useful for the identification and strain typing of a variety of bacteria. We describe a patient with Salmonella enterica serotype Enteritidis infection involving a total knee arthroplasty. Total nucleic acids were extracted from the joint fluid of the patient. A portion of the 16S rDNA gene was amplified using broad-range PCR primers and a sequence was derived using pyrosequencing. After we discovered the identification of the microorganism from the bacterial DNA, we also evaluated two Salmonella enterica strains available in our clinical microbiology laboratory by pyrosequencing.

The case

The patient was a 71-year-old woman who had developed pain in both knees 2 weeks prior to her visit to our emergency department. Her past medical history revealed rheumatoid arthritis (RA) of 12-years duration, which had been treated with 5 mg prednisone, 100 mg azathioprine, 400 mg hydroxychloroquine, and 15 mg/week methotrexate in the past. The patient had undergone total knee arthroplasty in both knees (right knee, 11 years previously; left knee, 6 years previously), and total hip arthroplasty (11 years previously).

She described severe pain, swelling, painful movement, stiffness, and effusion. The patient did not recall any injuries. Physical examination revealed tenderness to palpation, swelling, increased warmth, and limited range of motion in both knees. Her temperature was 37.0 °C. Laboratory studies revealed: white blood cell (WBC) count $7.2 \times 10^9/l$, with 88% neutrophils. Left knee joint aspiration produced cloudy, slightly bloody-yellow fluid with $121 \times 10^9$ neutrophils. Gram stain did not reveal any microorganisms. A blood culture showed no growth. The pre-operative plain radiographs of both knees were unremarkable. The patient was admitted, and treated with ciprofloxacin therapy since her operation (2 years previously), and total hip arthroplasty (11 years previously).

The patient had undergone total knee arthroplasty in both knees. Her temperature was 37.0 °C. Laboratory studies revealed: white blood cell (WBC) count $7.2 \times 10^9/l$, with 88% neutrophils. Left knee joint aspiration produced cloudy, slightly bloody-yellow fluid with $121 \times 10^9$ WBC/l, with 92% neutrophils, and $8.3 \times 10^9$ red blood cells/l. Gram stain did not reveal any microorganisms. A blood culture showed no growth. The pre-operative plain radiographs of both knees were unremarkable. The patient was admitted, and treatment consisting of intravenous ciprofloxacin 400 mg was initiated.

After day 2 of hospitalization, the joint fluid culture grew out only a few colonies of Salmonella enterica serotype Enteritidis. Open irrigation and debridement were performed and the polyethylene liners were replaced in both knees. Intraoperative cultures from tissue of both knees and from synovial fluid of the left knee grew Salmonella sp.

Postoperatively, the patient’s pain was well controlled, and the wounds were clean, dry, and intact. Abdominal computed tomography showed intra- and extra-hepatic biliary dilatation and a cyst in the kidney. There were no abscesses or any positive findings that could identify the source of the Salmonella. The results of an endoscopic retrograde cholangiopancreatography (ERCP) confirmed that the gallbladder was not the source of Salmonella sp.

The patient recovered from surgery and was discharged home in stable condition. At 6 weeks postoperatively, the surgical wounds had healed. Although there was warmth in both of her knees, the joint fluid culture aspirated from the right knee was negative. There was still warmth in both of her knees at 12 weeks postoperatively, but the swelling and erythema had resolved. She was able to ambulate using a cane outside her house and without a cane inside. She has been treated with ciprofloxacin therapy since her operation without change in symptoms with 30 months of follow-up.

Materials and methods

Nucleic acid isolation and real-time PCR

The joint fluid aspirated at her visit to the emergency department was stored for 5 days at −20 °C until DNA extraction. DNA extraction was performed using MagNA Pure LC DNA Isolation Kit III on a MagNA Pure instrument (Roche Diagnostics, Indianapolis, IN, USA). Considering the identification of the isolate from the culture, we also isolated the DNA from two laboratory strains of Salmonella enterica serotype Enteritidis using a rapid boiling procedure. Aliquots from the extracted DNA were then evaluated by real-time PCR using the Rotor-Gene 3000 (Corbett Research, Australia). The PCR assay employed broad-range PCR primers that targeted a portion of the 16S rDNA gene.

The primers (BioChem, Salt Lake City, UT, USA) were: forward primer, 5'-biotin-GCACAAGCGGTGGAGC-3'; reverse primer, 5'-GGGACTTACCCCAAAAC-3'. The forward primer was covalently coupled to biotin at the 5' end to obtain a sequencing template from the PCR product. The PCR mixture consisted of 4.0 mmol/l MgCl$_2$, 0.2 μmol/l of each primer, 12.5 μl of 2 × SensiMix (Quantace, Norwood, MA, USA), and 0.5 μl of 50 × SYBR green I (Quantace) for a volume of 20 μl master mix. Five microliters of template DNA extract were added to the reaction mixture, for a final reaction volume of 25 μl for each tube. The cycling condition was 95 °C for 10 min, followed by 45 cycles of denaturation at 95 °C for 10 s, annealing at 52 °C for 30 s and 72 °C for 15 s.

Pyrosequencing

Pyrosequencing was performed as recommended by the manufacturer. Twenty microliters of each biotinylated PCR product was combined with a streptavidin bead—buffer mix and transferred to a 96-well filter plate. Vacuum was applied to remove solution, and the beads were incubated with denaturation solution. After two washes, beads were vacuum dried and resuspended in annealing buffer. Forty microliters of the beads/buffer solution containing the single-stranded template was transferred to a PSQ 96-well plate containing 4 μl of 4 μmol/l sequencing primer and incubated for 2 min at 90 °C. The sequencing primer was 5' - CCATGCGACGACCTGT - 3'.

The pyrograms were analyzed using standard pyrosequencing software, and the first 30-bp sequence was submitted as Basic Local Alignment Search Tool (BLAST, http://www.ncbi.nlm.nih.gov/blast/Blast.cgi) queries through the National Center for Biotechnology Information (NCBI, http://www.ncbi.nlm.nih.gov/).

Results

The quantification curve from the DNA extract demonstrated the presence of bacterial DNA (Figure 1A). The pyrogram of the sample from this patient and from the two Salmonella isolates in our laboratory showed the same sequence: CTC ACA GTT CCC GAA GGC ACA AAT CCA TCT (Figure 1B). This 30-bp sequence corresponds with the sequence of Salmonella enterica by BLAST analysis of GenBank.
Discussion

Non-gonococcal bacterial arthritis usually presents with abrupt onset in a single joint. Dubost et al. reported that polyarticular septic arthritis occurs in 16.6% of all septic arthritis cases and tends to occur in patients with RA or immunosuppression caused by drugs or by concurrent illness. Some authors have reported cases of bilateral septic knee arthritis due to Salmonella enterica serotype Enteritidis, which developed in patients with systemic lupus erythematosus (SLE) or Hodgkin’s lymphoma. One of the authors reported that 59% of patients with systemic SLE in the Salmonella sp infection group had polyarticular involvement. The patient reported here had RA and had previously undergone total knee arthroplasty in both knees. Joint pain in patients with inflammatory arthropathies is often interpreted as an exacerbation of the underlying condition, so it is appropriate to consider the possibility of septic arthritis in patients with chronic joint disease.

Infections that occur later than 3 months after joint arthroplasty are usually hematogenously acquired. The usual pathogenesis of Salmonella septic arthritis is thought to be hematogenous rather than direct inoculation into the joint. The patient described in this report did not present with any gastrointestinal symptoms, and imaging studies did not identify a source of Salmonella sp, but hematogenous seeding of the knees still seems likely. The fact that the patient had bilateral knee infections simultaneously also supports hematogenous spread, even though the primary source of bacteremia was never identified.

The treatment of prosthetic joint infection commonly requires the surgical removal of all bioprosthetic components, although high-risk patients have been treated with long-term suppressive antimicrobial therapy. Day et al. reviewed 12 patients with prosthetic joint infections due to Salmonella sp including the patient they reported. Nine of the 12 patients with a Salmonella sp prosthetic joint infection required prosthesis removal. Three patients with retention of the prosthesis were treated with suppressive antibiotic therapy. One patient experienced recurrence of infection after two and a half years. Day et al. and Musante and Ogden reported that their patients had a successful outcome with removal and immediate replacement of one component of the joint. For the current case, the decision was made to perform an open irrigation and debridement on both knees and to replace the polyethylene liners.

Although the molecular identification was retrospectively performed in this case, broad-range PCR assays, particularly those that target the 16S rDNA gene, are of interest because of the potential for accurate and rapid detection of the bacterial genome, and have been used to detect of a wide variety of bacteria. Several authors have used pyrosequencing as a method of bacterial identification. In this report, we have described the successful application of pyrosequencing to the identification of Salmonella sp directly from joint fluid, although Gram stain was negative and culture results demonstrated only few bacteria. In the future, broad-range PCR followed by DNA sequencing may be used as an adjuvant highly sensitive means to identify the etiologic agents of bacterial infections.

Figure 1  A Quantification mode of real-time PCR. Two Salmonella enterica serotype Enteritidis samples in our laboratory (*) were amplified at around 10 cycles. The amplification of the clinical sample DNA occurred at 16 cycles. (B) The pyrogram of the case. This pyrogram is read as follows, “CTCACAGTTC……”. This sequence was categorized as Salmonella enterica according to BLAST.
Conclusion

Salmonella sp is an uncommon cause of joint infection, and bilateral Salmonella enterica serotype Enteritidis infection of total knee arthroplasty is rare. To the best of our knowledge, this is the first report of detection of Salmonella sp in joint fluid using universal PCR followed by pyrosequencing.

Conflict of interest: No conflict of interest to declare.

References