Direct Detection of Indirect Transmission of *Streptobacillus moniliformis* Rat Bite Fever Infection

Running Title: Direct detection of *Streptobacillus* Infection

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Abstract:
We describe the evaluation of culture negative synovial fluid from a 3-year-old boy by PCR and electrospray-ionization followed by mass spectrometry (PCR/ESI-MS). Our patient developed a diffuse rash and fever with systemic signs and symptoms of sepsis but four sets of blood cultures obtained prior to initiation of antibiotics were negative. After one week of illness he developed right knee swelling. Analysis of synovial fluid was consistent with infection, but cultures of specimens obtained following initiation of antimicrobial treatment were negative for growth. PCR/ESI-MS detected *Streptobacillus moniliformis* in the synovial fluid sample. Our patient completed an appropriate course of antibiotic treatment and remained completely asymptomatic in follow-up evaluation. This unique case suggests PCR/ESI-MS may be a useful diagnostic tool for direct detection of unusual or unexpected pathogens directly from clinical specimens, particularly when samples have been obtained from patients following initiation of antibiotic therapy.

Case Report:
A previously healthy 3-year-old boy who was at 85th percentile in height and weight and was current on all ACIP recommended immunizations presented for evaluation of fever and a rash. His mother reported a three-day history of fever, irritability, poor intake, emesis, and a rash that began as a papulovesicular rash on the forearms. He became progressively irritable over the following three days and began to complain of left leg pain. On the day of admission the rash had spread to the face, neck, legs, hands and feet, including palms and soles, and he was unable to tolerate walking or standing on his left leg due to pain.

Upon presentation the patient was febrile and tachycardic. The exam was notable for several 1-2 mm vesicular lesions in the posterior pharynx as well as erythematous papular 2-4 mm vesicles on the arms, legs, buttocks, palms, soles, ears, and cheeks. The left hip was tender to palpation and painful with...
both active and passive range of motion, but no erythema, swelling or edema was noted. Urinalysis, complete metabolic profile and blood counts were all normal, but the C-reactive protein (CRP) was markedly elevated at 15.24 mg/dL.

After three sets of blood cultures were obtained in the emergency department (ED) the patient was hospitalized for further testing which included urine culture, throat culture for *Streptococcus pyogenes*, a nasopharyngeal swab for respiratory viral cultures, and one additional blood culture (all cultures were ultimately negative); as well as EBV, CMV and *Leptospira* serologies, which were also all negative. During the patient’s hospital course his fever persisted with daily core temperatures exceeding 39 °C (102.2 °F), and the erythematous papular rash migrated throughout the surface of his body (see Figure 1). On hospital day four, he developed a right knee effusion and the CRP had increased to 19.31 mg/dL. Further questioning of the family revealed the boy had suffered a minor laceration of his right index finger from the cage where his grandmother keeps her pet rat one week prior to the onset of symptoms. He had no direct contact with the rat. Ampicillin 800 mg IV every six hours was started empirically for suspected rat bite fever (RBF). On hospital day eight, following two doses of IV ampicillin, a right knee arthrocentesis was performed in standard fashion. Cloudy, yellow synovial fluid was obtained that was notable for WBC count of 62,415 cells/mL (91%PMNs). Gram stain and cultures were all negative. The microbiology laboratory staff was notified of our concern for RBF and the specimen was inoculated onto trypticase soy agar and cultures were held for 30 days. In addition to septic arthritis, endocarditis is also a well described complication of RBF; blood cultures were also held for 30 days.

The patient was treated with ampicillin 800 mg IV every 6 hours for 6 days. He gradually responded to treatment. Rash, right knee swelling and left hip pain all improved. On the morning of March 27, 2014...
hospital day 10, the CRP had decreased to 2.92 mg/dL and the patient was discharged home with a plan to complete an additional 7 days of oral amoxicillin. A follow-up CRP one week following completion of antibiotic treatment was 0.20 mg/dL.

Even with common pathogens, reliance on culture for identification of bacteria in synovial fluid is problematic. In clinical practice, the sensitivity of synovial fluid culture is roughly 80% and is significantly diminished by prior empiric antibiotic treatment and/or the presence of organisms not detected by standard culture techniques. Detection of common bacterial pathogens by PCR can be useful, but requires correct anticipation of expected organisms. PCR/ESI-MS has demonstrated utility in detection of pathogenic bacteria directly from specimens obtained following initiation of antimicrobial treatment and is not predicated on prior anticipation of the most likely pathogen. (3)

Institutional IRB approval was obtained for submission of samples of our patient’s serum and right knee synovial fluid for testing by PCR/ESI-MS, as described by Kaleta et al. (4) Compared to clinical samples, the assay performs with 98.7% and 96.6% concordance at the genus and species levels, respectively. PCR/ESI-MS detected Streptobacillus moniliformis in synovial fluid at a high level of detection (255 genome equivalents per PCR reaction well), but did not detect an organism in peripheral blood, or BacTec™ blood culture bottles. Our failure to isolate this organism in culture despite inoculation of appropriate media is not surprising. Even with appropriate media, and without prior antibiotic exposure, S. moniliformis does not reliably grow in culture. The PCR/ESI-MS identification was confirmed by partial 16S ribosomal RNA gene sequencing using Clinical Laboratory Standards Institute MM18-A M13-tagged bacterial 16S rDNA primers with a single base modification in the reverse primer and M13 tags added to the 5’ end. The primer sequences:
The aligned trimmed 708 bp sequence (with primers removed):

AGGATGAACGCTGACAGAATGCTTAACACATGCAAATCTATGTATGAAATGTAAGCTTG
CTTACATAGACTACATGGTGGAAGTTGTGAAGTAAGGAAACCTACCTTTAGACT
GGGATAACCATTAGAATATGATGATAATATGATAGATAGGACGCTACTACTA
TTATGAAAGGAGAGATGGCTAAGGAGAGAGGCTAATGGGATATGGAACATG
GAGGAACCTCTGATCACTGAAATGCTTGCGAAGAAGGTGTTTTTAGGTGTTAACGT
CTTCTAGGGGAGGAAGAAGAAGATGACGTAATCTACAGAAGACGCAGCCTAATACG
TGCCAGACGCGGTAATACGTATGTCGAACGGAAGGTGTTTTTCGAATATGGG
GGGATAACCATTAGAATATGATGATAATATGATAGATAGGACGCTACTACTA
TTATGAAAGGAGAGATGGCTAAGGAGAGAGGCTAATGGGATATGGAACATG
GAGGAACCTCTGATCACTGAAATGCTTGCGAAGAAGGTGTTTTTAGGTGTTAACGT
CTTCTAGGGGAGGAAGAAGAAGATGACGTAATCTACAGAAGACGCAGCCTAATACG
TGCCAGACGCGGTAATACGTATGTCGAACGGAAGGTGTTTTTCGAATATGGG

An NCBI Blast query of our 708 bp partial 16S sequence against GenBank reference bacterial and Archaea 16S ribosomal RNA gene sequences exhibited 100% identity to Streptobacillus moniliformis DSM 12112 16S ribosomal RNA complete gene sequence, positions 19 to 726 Escherichia coli numbering system (GenBank sequence ID NR_074449.1).

S. moniliformis, the primary agent of RBF in North America, is a pleomorphic, filamentous, gram negative, non-motile bacillus. It is extremely fastidious, requiring microaerophilic conditions to grow in culture. Confirmation of suspected RBF is of critical clinical importance as sequela from undiagnosed cases include: endocarditis, pericarditis, arteritis, volvulus, septicemia, bronchitis and pneumonia; and untreated RFB has a mortality rate of 10%. (5) Our patient had knee pain, a palpable knee effusion, and a persistently elevated CRP with negative cultures and serologic testing; prompting further evaluation of right knee fluid. Although our patient had no direct contact with mice or rats, the clinical symptoms and source of the finger laceration provoked suspicion of RBF. Yet antibiotic pre-
treatment may have been responsible for negative cultures, so we were concerned about common
bacterial pathogens as well. Uncommon pathogens detected by PCR/ESI-MS in synovial fluid have
been reported, but this is the first report of disseminated RBF detected by PCR/ESI-MS. (6)

Clearly, the approach to culture-negative infections and our understanding of syndromes caused
by pathogens that cause disseminated disease but are not readily diagnosed by conventional
microbiologic methods requires further study. Nonetheless, our experience in this case suggests that
molecular methods capable of timely identification of unusual or unanticipated bacteria directly from
synovial fluid may provide an opportunity to detect virulent pathogens and direct antibiotic treatment;
and that PCR/ESI-MS may have a role as an adjunct to conventional diagnostic microbiologic methods
in culture-negative synovial fluid specimens.

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References:
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Figure 1. Representative maculopapular exanthema on right thigh and left thigh and buttocks – day 5.