Case Report

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Septic arthritis due to *Roseomonas gilardii* in an immunocompetent adolescent

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The genus *Roseomonas* comprises groups of slow-growing, Gram-negative coccobacilli, which only infrequently cause infection in humans. When identified, they are associated with immunocompromised adults, often causing bacteraemia. Due to their rarity, members of this genus can be overlooked or misidentified using automated laboratory identification systems. We report on an immunocompetent adolescent patient who developed septic arthritis due to *Roseomonas gilardii* following surgery for a sports injury. The isolate was initially misidentified as *Bordetella bronchiseptica* using the Vitek 2 system, but confirmed as *R. gilardii* based on 16S rRNA gene sequencing. To the best of our knowledge, this is the first case of a healthy paediatric patient with septic arthritis due to *R. gilardii*.

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Introduction

Roseomonas gilardii is a member of the bacterial genus *Roseomonas*, a group of slower-growing, Gram-negative bacteria (Rihs *et al.*, 1993). These coccobacilli are generally uncommon as causes of human infection, but have been reported in some case series in immunocompromised hosts (Dé *et al.*, 2004). Cases of disease in children are exceedingly rare (McLean *et al.*, 2006), as are infections involving the bones and joints.

Due to the rarity of this infectious agent, it may be overlooked from a clinical and microbiological perspective. A method to identify organisms collected from sterile sites, such as the Vitek 2 system (bioMérieux), might not correctly identify *Roseomonas* species. For such species after several days growth in an appropriate culture environment, characteristic pink, mucoid colonies are seen (Shokar *et al.*, 2002).

In this paper we report a case of previously healthy girl with septic arthritis of the knee due to *R. gilardii*, following surgical repair of a sports injury. Interesting features of this case include that the isolate was initially misidentified as *Bordetella bronchiseptica*, with subsequent corrected identification based on 16S rRNA gene sequencing, and that, to the best of our knowledge, this is the first reported case of septic arthritis due to *R. gilardii* in an immunocompetent paediatric patient.

Abbreviations: PICC, peripherally inserted central catheter; PMN, polymorphonuclear leukocyte; TMP–SMX, trimethoprim–sulfamethoxazole.

Images of *R. gilardii* grown on different agars are available as supplementary data with the online version of this paper.

Case report

A 16-year-old, unimmunized, previously healthy female, 11 months after sustaining a sports-related right anterior cruciate ligament injury, underwent reconstructive surgery, with an autologous hamstring tendon graft implanted with two biodegradable screws. Two weeks post-operatively she developed malaise, right knee swelling and fever, but did not seek medical attention. Symptoms persisted, and 1 week later she was diagnosed at a walk-in clinic with an *Escherichia coli* urinary tract infection and treated for 1 week with trimethoprim–sulfamethoxazole (TMP–SMX). After the TMP–SMX treatment was completed (28 days post-operatively) she was seen by her surgeon for ongoing malaise and knee swelling, and was started on a treatment of oral cloxacillin with TMP–SMX. A clinical description of the knee was unavailable as the patient was seen at an outside facility. The joint was not aspirated at that time.

Thirty-two days post-operatively she was again seen by her surgeon because of ongoing symptoms. Joint aspiration showed red, turbid fluid with a cell count of 3.8×10^{10} nucleated cells l^{-1} , and a Gram stain showed 2+ polymorphonuclear leukocytes (PMNs) (1–5 PMNs per high-power field), but no organisms, and a culture was negative. At 38 days following surgery, her symptoms had not resolved so the joint was flushed and sampled again. The cloxacillin and TMP–SMX were discontinued. Daily treatment with 1 g ceftriaxone through a peripherally inserted central catheter (PICC) was started. Repeat culture and Gram stain of the synovial fluid were negative. Biochemical analysis was not performed. Her systemic and joint symptoms began to improve.

Five days later she presented to the Pediatric Emergency Department, Winnipeg Children's Hospital, Winnipeg, Manitoba, Canada, with renewed fever, malaise and headache, and with erythema and tenderness over the PICC insertion site with proximal extension. On admission, her temperature was 37 °C and she had stable vital signs. A moderate right knee effusion was noted as well, with a decreased range of motion, but no pain, erythema or evidence of superficial infection. Her white blood cell count was 8.7×10^9 cells l^{-1} with 5.1×10^9 neutrophils l^{-1} . Her haemoglobin level was 119 g l^{-1} , her platelet level was 352 000 platelets mm^{-3} and she had an erythrocyte sedimentation rate of 66 mm h^{-1} . Knee X-rays showed a moderate effusion, but were otherwise normal. A clinical diagnosis of thrombophlebitis was initially made and the PICC line was removed. Intravenous cefuroxime treatment was started. Blood cultures and cultures of the PICC tip were negative. The next day, an ultrasound of the arm showed a cephalic vein clot that extended from the antecubital fossa to the shoulder.

One week after admission, her symptoms of right knee swelling, decreased range of motion and inability to bear weight suddenly worsened. Needle aspiration of the joint showed no PMNs; the synovial fluid grew a *Bacillus* sp., which was considered a contaminant. A day later she was taken to the operating theatre for drainage of the knee. A Gram stain showed 3+ PMNs (5–10 PMNs per high-power field) and no bacteria. Culture from the open lavage grew small (<1 mm) grey colonies of a single morphotype that were short Gram-negative bacilli in pairs and short chains after 48 h incubation on blood agar, chocolate agar and MacConkey agar. The organism was initially reported as *B. bronchiseptica* based upon identification by a Vitek 2 system (bioMérieux). A colorimetric card was used, Gram Negative Identification 21341, with 'excellent' identification. The antibiotics were changed to 2 g ceftriaxone once a day and 100 mg oral doxycycline once a day, with clinical improvement.

Given the lack of clinical correlation in the literature between *B. bronchiseptica* and septic arthritis, the laboratory workup of the isolate was pursued further. On day 3 of culture incubation, the colonies developed a light pink pigment indicating that the initial identification of the isolate was incorrect. Culture on Sabouraud agar followed by growth of large (>6 mm) mucoid pink colonies, growth on blood agar growth at 42 °C, a weak oxidase-positive reaction, a urease-positive reaction and a lack of fluorescence following UV light exposure identified the organism as a *Roseomonas* species. On the basis of 16S rRNA gene sequencing, the isolate was subsequently identified as *R. gilardii* (100% identity by BLAST search). The primers used spanned nucleotides 10–805 (based on *E. coli* sequence) (giving an amplified fragment of 795 bp) of the 16S rRNA gene, covering hypervariable regions 1–4. Antimicrobial susceptibility testing was performed by a custom-made broth microdilution using cation-adjusted Mueller–Hinton broth according to Clinical and Laboratory Standards Institute recommended methods

[M7-A7 2006 and M100-S17 2007 (CLSI, 2006, 2007)] giving MICs of 0.12 mg l^{-1} for gentamicin, 1 mg l^{-1} for minocycline, 0.50 mg l^{-1} for ciprofloxacin, >32 mg l^{-1} for cefotaxime, >256 mg l^{-1} for ceftazidime and >256 mg l^{-1} for amoxicillin–clavulanate. An Etest (bioMérieux) was also carried out, giving MICs of 0.75 mg l^{-1} for minocycline, >32 mg l^{-1} for cefotaxime, 0.125 mg l^{-1} for gentamicin, >256 mg l^{-1} for amoxicillin–clavulanate, 0.5 mg l^{-1} for ciprofloxacin and >256 mg l^{-1} for ceftazidime. No TMP–SMX data were available. The patient was continued on 100 mg oral doxycycline twice a day as monotherapy with improvement, and was discharged home.

A follow up 1 month later showed she had made good progress, with a significantly decreased effusion and no changes consistent with osteomyelitis on plain films. A magnetic resonance imaging scan done at this time showed possible evidence of bone marrow oedema. Blood work showed she had a normal erythrocyte sedimentation rate and white blood cell count. She still had knee pain when ambulating long distances. Her course of doxycycline was extended for 2 more weeks, giving a total of 8 weeks treatment. At a follow up 2 weeks later, the patient was asymptomatic, plain films were normal and her antibiotics were discontinued.

Discussion

The bacterial genus *Roseomonas* encompasses four species (*R. gilardii*, *Roseomonas mucosa*, *Roseomonas cervicalis* and *Roseomonas fauriae*), and was first classified by Rihs and colleagues in 1993 (Rihs *et al.* 1993). These slow-growing, aerobic Gram-negative coccobacilli are oxidase positive, and produce a pink pigment (Steinberg & Del Rio, 2005). Mucoid colonies are seen occasionally, especially with the almost runny appearance of *R. mucosa* (Han & *et al.*, 2003).

Roseomonas sp. can cause a variety of clinical diseases including: bacteraemia (Dé *et al.*, 2004), soft-tissue infection (Shokar *et al.*, 2002), and bone or joint infection (Nahass *et al.*, 1995; Sipsas *et al.*, 2006). A case series of 36 cancer patients with *Roseomonas* bacteraemia or catheter-associated infection showed approximately 60% of cases were due to *R. mucosa* and 22% were due to *R. gilardii* (Dé *et al.*, 2004). Symptoms were noted in ~80% of patients, with fever being the most common (~75%). Other infections with *Roseomonas* sp. are generally associated with some degree of immunosuppression or other chronic conditions (Steinberg & Del Rio, 2005).

Very few cases of *Roseomonas* infection have been reported in the paediatric literature, with only seven described to date (McLean *et al.*, 2006). Two cases reported only transient colonization and another included little clinical information (Struthers *et al.*, 1996). Two cases of *R. gilardii* bacteraemia in the setting of acute lymphoblastic leukaemia with full recovery were reported (Struthers *et al.*, 1996; McLean *et al.*, 2006). A 3-month-old infant with stage 3 neuroblastoma was reported with a port-associated mixed *Klebsiella oxytoca* and *R. fauriae* bacteraemia (McLean *et al.*, 2006).

Bone and joint infections appear to be a less frequent presentation of infection with *Roseomonas* sp., although osteomyelitis has been reported (Nahass *et al.*, 1995). A recent case report from Greece (Sipsas *et al.*, 2006), described the first documented case of a septic arthritis due to *R. mucosa*. Similar to other presentations of *Roseomonas* infection, that patient was immunocompromised, with underlying rheumatoid arthritis, and treated with methotrexate and the anti-tumour necrosis factor alpha product (infliximab). Until now, to the best of our knowledge, there has been no documentation in the literature of either a paediatric or an immunocompetent patient with septic arthritis due to *Roseomonas*. Possible explanations include introduction of the organism at time of surgery, or at one of the subsequent outpatient knee aspirations. Clinical improvement was not seen until doxycycline treatment was used, supporting *Roseomonas* as the underlying aetiology.

In our case, the preliminary culture results from the knee aspirate were reported as *B. bronchiseptica*, but later confirmed as *R. gilardii* on the basis of biochemical testing and 16S rRNA gene sequencing. Several factors can account for this misidentification. First, the culture from the open lavage was identified by the Vitek 2 system (bioMérieux). The identification cards used by the Vitek 2 system do not identify the *Roseomonas* species, so the initial result was incorrect. Morphology, biochemical tests and 16S rRNA gene sequencing are the methods of identification for this species. Colonies appear small and raised, and are often mucoid and pink coloured (Han *et al.*, 2003; Shokar *et al.*, 2002). The pink colour often does not develop until after 3–4 days of growth on blood agar or chocolate agar (Supplementary Figs S1, S2 and S3 available with the online journal). It is possible that this was initially overlooked at the time the misidentification as *B. bronchiseptica* was made. The sequencing was pursued after the identification of *B. bronchiseptica* since the organism seemed inconsistent with the clinical presentation. Even though the quality of identification of *B. bronchiseptica* was excellent, the clinical isolate from the patient was very unreactive with the biochemical tests on the card, other than urea, so the initial identification was not believed. The features shared by *B. bronchiseptica* and *Roseomonas* are that both organisms are Gram-negative coccobacilli, and are oxidase-, catalase- and urease-positive (Woolfrey & Moody, 1991; Ner *et al.*, 2003; Mattoo & Cherry, 2005). Our patient had been receiving intravenous antibiotics prior to her knee aspiration, so growth of the organism in culture would not have been optimal.

Antibiotic treatment of *Roseomonas* can be challenging due to the high risk of resistance (Han *et al.*, 2003). Generally, *Roseomonas* sp. are sensitive to aminoglycosides, tetracyclines, ciprofloxacin and imipenem (Steinberg & Del Rio, 2005; Han *et al.*, 2003), and resistance is usually seen with penicillins, cephalosporins and TMP–SMX.

In summary, this is believed to be the first report of a case of septic arthritis due to *R. gilardii* in an immunocompe-

tent paediatric patient, following knee surgery, with initial misidentification as *B. bronchiseptica* based on results from the Vitek 2 system and subsequently corrected via molecular testing. Further studies should be pursued when automated systems yield results not consistent with clinical scenarios. This organism is an uncommon pathogen in the healthy paediatric population.

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