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Molecular Diagnosis of *Kingella kingae* Pericarditis by Amplification and Sequencing of the 16S rRNA Gene▽

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***Kingella kingae* is a fastidious gram-negative bacillus that is considered an emerging pathogen in pediatric settings but remains less common in adults. Here we describe a case of pericarditis in an immunocompetent adult host. The microorganism was identified directly from the clinical sample by molecular techniques, i.e., 16S rRNA gene amplification and sequencing.**

CASE REPORT

In December 2006, a 43-year-old woman was admitted to our hospital for dyspnea and fever. The patient had no significant medical history. A week before admission, she had complained of fever with a sore throat and was treated with 1 g of amoxicillin (three times a day) for 5 days without improvement. She was then admitted for dyspnea. The clinical exam was unremarkable except for a temperature of 38.2°C and reduced breath sounds in the left lung base. The leukocyte count was 16,300/mm^3 (absolute neutrophil count, 11,200/mm^3), and the hemoglobin level was 10 g/dl. The C-reactive protein (CRP) was markedly elevated at 308 mg/liter (normal level, <10 mg/liter), and the liver enzyme levels were twice the normal range. The patient was hypoxic, with an arterial partial pressure of oxygen equal to 59 mm Hg. A chest X-ray showed enlargement of the cardiac silhouette, and an elevation of the ST segment (the time between the end of the ventricle's depolarization and the beginning of repolarization) compatible with pericarditis was noted on the electrocardiogram. Transthoracic echocardiography was rapidly performed and revealed a circumferential pericardial effusion with tamponade.

The patient underwent emergency pericardiac drainage of 800 ml of pus, and a pericardial biopsy was performed at the same time. The anatomopathological examination of the pericardial tissue showed inflammation with altered neutrophils. Gram staining of the fluid and of the pericardial tissue showed no microorganisms, and cultures both on chocolate agar medium and in blood culture bottles (Hemoline DIPH-F; bioMérieux SA, Marcy l'Etoile, France) were negative.

The patient was treated empirically with amoxicillin-clavulanic acid, 1 g four times a day, intravenously. On the third day after drainage, the patient experienced exacerbation of the dyspnea associated with a left pleural rub and an increase in leukocyte count up to 30,000/mm^3. A chest computed-tomography scan showed a recurrence of pericardial fluid and a new bilateral effusion with passive atelectasis. A second surgical pericardial drainage was performed along with a bilateral pleural thoracentesis. Ten days later, the patient was still febrile and developed right heart failure, with the leukocyte count and CRP remaining elevated. Transthoracic echocardiography confirmed the presence of constrictive pericarditis and the persistence of a loculated pleural effusion. Treatment was then changed, and corticosteroids (methylprednisolone, 1 mg/kg intravenously) and doxycycline (100 mg twice a day) were added. Streptokinase was injected twice a day through a chest tube.

Since the patient had taken antibiotics prior to admission, 16S rRNA gene amplification and sequencing were performed directly from the pericardial fluid. DNA was extracted from the pus with a QIAamp tissue kit (QIAGEN, Chatsworth, CA). The universal prokaryotic primers p91E [5′-GGAATTCAAA (G/T)GAATTGACGGGGGC-3′] and p13B (5′-CGGGATC CCAGGCCCGGGAACGTATTCAC-3′) were used along with *Taq* polymerase (Sigma, Saint Quentin Falavier, France) to amplify a 475-bp segment corresponding to part of the 16S rRNA gene (10). The nucleotide sequences of both strands of the amplified DNA fragment were determined with a model 3700 DNA analyzer after application of the BigDye terminator cycle sequencing ready reaction (Applied Biosystems, Foster City, CA). Similarity searches were carried out against GenBank and the Ribosomal Database Project (RPDII; Michigan State University). It showed the presence of *Kingella kingae* genetic material in the pericardial fluid with 100% identity to reference strain ATCC 23330. Doxycycline was then stopped, and a course of 4 weeks of amoxicillin-clavulanic acid was completed with 2 weeks of prednisone at a dose of 1 mg per kg of body weight with progressive tapering.

The patient's recovery was complete, and all the drains were removed 2 weeks later. The patient was discharged after 29 days of hospitalization with a normal leukocyte count and a normal CRP.

Discussion. *Kingella kingae* is an aerobic, short, gram-negative bacillus. It is oxidase positive and catalase negative, has a

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selective acid production from glucose and maltose, and produces a beta-hemolytic reaction when grown on blood agar (16).

Since its discovery in 1960, *K. kingae* has been considered a rare cause of endocarditis in adults and is included in the HACEK group (i.e., the group of gram-negative bacilli comprising *Haemophilus parainfluenzae*, *Haemophilus aphrophilus*, *Haemophilus paraphrophilus*, *Actinobacillus actinomycetemcomitans*, *Cardiobacterium hominis*, *Eikenella corrodens*, and *Kingella* species) (8). However, with the development of molecular techniques, most notably 16S rRNA gene PCR (12), and the incubation of joint aspirates in blood culture vials for enhancing microbiological recovery (19), it is increasingly identified as a pathogen. In pediatric settings (18), *K. kingae* colonizes the respiratory and oropharyngeal tracts of almost 4% of toddlers, causing primarily septic arthritis and bacteremia in children younger than 4 years old (21). In this population, it is considered the second most common cause of septic arthritis, with almost 12% of documented cases of arthritis caused by this pathogen (6). A few other reports in pediatric settings include discitis, osteomyelitis, and infections of the central nervous system (1, 5, 13).

In adults, *Kingella kingae* is an uncommon cause of infection and occurs primarily in immunocompromised hosts suffering from AIDS, lupus, or other vasculitis (4, 17); however, it has also been described as a cause of arthritis, endocarditis, meningitis, and bacteremia in immunocompetent hosts (3, 9, 11). To our knowledge, no case of pericarditis caused by *K. kingae* has been described to date.

No clear portal of entry was identified in our patient, but the history of sore throat could be related to the oropharyngeal carriage of the organism. No culture was performed to confirm this assumption, since the patient had taken amoxicillin for 5 days before admission. After the results of the molecular diagnosis were obtained and although most *K. kingae* isolates are susceptible to penicillin (20), the treatment was not switched to amoxicillin alone, since no bacteria could be isolated and some strains that are resistant to this antibiotic due to production of penicillinase have been reported (15).

Because of the persistence of the effusion and the occurrence of a constriction, corticosteroids were added to the antimicrobial regiment. It was expected that the corticosteroids could provide an anti-inflammatory effect which could decrease the pericardial constriction as occurs in patients with tuberculous pericarditis or bacterial meningitis (2, 7). There are also some reports of success using fibrinolysis in a nontuberculous setting (14). In our patient, we could not assess with certainty the positive effect of corticosteroids; nevertheless, their use in treating constrictive bacterial pericarditis should be further investigated.

We should remember that although *K. kingae* is an emerging pathogen in pediatric settings, it can also occur in adult hosts, potentially causing invasive diseases. It is also a fastidious organism, and molecular diagnosis is useful for its identification directly from clinical samples, particularly in cases of prior antibiotic therapy.

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