Therapy with penicillin and ceftriaxone produced no clinical improvement in his condition. Treatment with isoniazid, rifampin, ethambutol, and pyrazinamide was begun for possible tuberculous meningitis; his condition improved, and he was discharged.

Two weeks later, he was readmitted with obtundation and nuchal rigidity. Examination of the CSF showed a WBC count of 1,260/μL (100% neutrophils), a protein level of 4.6 g/L, and a glucose concentration of 1.4 mmol/L; gram stains and stains for AFB and a cryptococcal antigen assay were again negative. It was now recognized that previous cultures had been positive for M. fortuitum, and amikacin and doxycycline were administered. The patient died 3 days later.

At autopsy, the brain weighed 1,200 g, and basilar meningitis was noted (figure 1); histopathologic findings included neutrophils with rare multinucleated giant cells but no granulomas. Gram stains and stains of tissue for AFB showed branching extracellular AFB and gram-positive bacilli. A culture yielded M. fortuitum.

M. fortuitum infection rarely involves the CNS; only four cases of meningitis due to this organism have been reported [3–6]. Three of these cases occurred in immunocompetent patients and resulted from spread of a local infection (i.e., mastoiditis, a posttraumatic sacral abscess, and a cauda equina abscess) [3–5]. The fourth case involved a 28-year-old patient with AIDS who presented with pleuritic pain, fever, a productive cough, headache, and vomiting; however, this patient was lost to follow-up shortly after presentation [6].

Our case is instructive for several reasons. M. fortuitum stains poorly with fluorochrome stains and therefore may not be recognized in smears of CSF that have been stained with fluorochrome; in addition, because M. fortuitum is positive on gram staining, it may be mistaken for pleomorphic gram-positive bacilli such as diphtheroids. Use of a carbolfuchsin stain is often necessary when donovanosis, but the sensitivity of these histological techniques varies between 60% and 80% [1].

Kharsany et al. recently reported the isolation of C. granulomatis in a monocyte coculture system and suggested the development of molecular-based diagnostic methods for the detection of C. granulomatis [2]. Other researchers have used PCR primers targeting conserved genes to identify noncultivable pathogenic eu- bacteria [3]. C. granulomatis has been linked with the Klebsiella species on the basis of common ultrastructural morphology, antigenic cross-reactivity, and similar pathogenicity (e.g., Klebsiella rhinoscleromatis is an intracellular organism that causes chronic destructive ulceration of the nasopharynx) [1, 4–6]. We therefore designed PCR primers targeting the phoE gene, which encodes a porin protein (i.e., membrane channel) and is conserved among Klebsiellae and other enterobacteria [7].

Biopsy specimens were obtained from six patients whose genital ulcers were clinically suggestive of donovanosis. DNA was extracted from one portion of each biopsy specimen, and the other portion was fixed and examined for Donovan bodies by the slow Giemsa (overnight) technique. DNA was also obtained from cultivated M. fortuitum isolates in the guidance of treatment.

Amplification of Klebsiella-Like Sequences from Biopsy Samples from Patients with Donovanosis

Donovanosis (or granuloma inguinale) is a genital ulcerative disease caused by a gram-negative bacillus, currently named Calymmatobacterium granulomatis. This disease is presumably sexually transmitted and is prevalent in India, southern Africa, South America, and Papua New Guinea as well as among Australian Aborigines [1]. C. granulomatis cannot be cultured with use of routine microbiological techniques and has been isolated by cultivation in the yolk sac of chick embryos on only 14 occasions [1]. Histological examination of smears or biopsy specimens from genital lesions therefore remains the principal laboratory method for detecting the causative organisms (i.e., Donovan bodies) of M. fortuitum is considered in the differential diagnosis. Moreover, isolates of M. fortuitum vary in their susceptibility to antimicrobial agents. Although most are susceptible to cefoxitin, cefmetazole, macrolides, sulfamethoxazole, doxycycline, and minocycline, our isolate was resistant to sulfamethoxazole and cefoxitin [7]. This finding reinforces the importance of susceptibility testing of M. fortuitum isolates in the guidance of treatment.

Michael B. Smith, Michael C. Boyars, and Gail L. Woods
Departments of Pathology and Internal Medicine, University of Texas Medical Branch, Galveston, Texas

References
Figure 1. Comparison of donovanosis PCR product with phoE sequences of Enterobacteriaceae. This unrooted phylogenetic tree is based on the 334-bp segment lying between the primers in the phoE PCR product. With use of the Clustal V multiple sequence alignment package, segments amplified from the samples obtained from patients with donovanosis, the syphilitic lesion, and three Klebsiella species were compared with published phoE sequences for Klebsiella oxytoca and other Enterobacteriaceae. Branch lengths are shown, as are the GenBank accession numbers for sequences obtained during this study and from the literature. NCTC = National Collection of Type Cultures.

<table>
<thead>
<tr>
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<th>Donovanosis (3 cases)</th>
<th>K. ozaenae NCTC 5051</th>
<th>K. pneumoniae NCTC 9633</th>
<th>K. rhinoscleromatis NCTC 5046</th>
<th>K. oxytoca X68022</th>
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<tr>
<td>U25350</td>
<td>0.03</td>
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mM MgCl₂, 0.01% gelatin, 0.2 mM of each deoxynucleoside triphosphate, 0.1 µM of each primer (i.e., 5'-ACCTACTGCAGG'TACCCGCTTTCTTCGG-3'; 5'-CAGGTCGGCCTCCTAACGTCTAACGCT-TG-3'), and 1 U of Taq DNA polymerase (Perkin-Elmer Cetus, Norwalk, CT). A 5-minute denaturation step at 94°C was followed by 40 cycles at 94°C for 1 minute, at 55°C for 1 minute, and at 72°C for 1 minute. Protocols for limiting and detecting PCR contamination were used. Products of the expected size (i.e., 385 bp) were purified, cloned, and sequenced.

The clinical diagnosis of donovanosis was confirmed for three of the six patients by histological demonstration of Donovan bodies in their biopsy specimens. PCR products of identical sequence were amplified on repeated occasions from these three biopsy specimens. The sequence of these PCR products was closely related to the phoE gene segments amplified from the K. pneumoniae, K. rhinoscleromatis, and K. ozaenae cultures but between 7.8% and 25.8% divergent from the corresponding sequences of other enterobacteria (figure 1).

Circumstantial evidence, including the previous association of C. granulomatis with Klebsiella species [1, 4–6], suggests that the Klebsiella-like sequence has been amplified from the donovanosis-causing organisms in these three specimens. One of the patients whose specimens were positive for Donovan bodies and for Klebsiella-like sequences by PCR was hospitalized, and extensive microbiological investigation of the biopsy specimen and of ulcer swabs failed to detect a contaminating enterobacterium to account for the amplification of a Klebsiella-like sequence from his genital lesion. In fact, Klebsiella species are unusual genital contaminants and have been isolated from only 1% of penile ulcers [8].

Donovan bodies could not be detected histologically in the biopsy specimens from the remaining three patients. One of these patients proved to have secondary syphilis. A 385-bp product was amplified from this patient’s specimen. However, sequencing found that this product was not Klebsiella-like but was instead homologous to the published phoE sequence for Escherichia coli (figure 1), suggesting that this product had been amplified from E. coli that was contaminating the syphilitic ulcer. Donovanosis remained the diagnosis for the final two patients whose biopsy specimens had all the histological features of donovanosis except for the presence of Donovan bodies. No PCR products were amplified from these Donovan body-negative biopsy specimens, although ethidium bromide-stained gel electrophoresis confirmed that high-molecular-weight DNA had been obtained from these specimens. PCR inhibitors may have been present, or the lesions may have contained only a small undetectable number of Donovan bodies. The PCR results for these latter three samples are therefore consistent with the suggestion that the Klebsiella-like sequence was amplified from the causative organism in the Donovan body-positive specimens.

In summary, we amplified a Klebsiella-like sequence from the genital lesions of three patients with donovanosis. In situ hybridization and in situ PCR experiments are in progress to confirm that this PCR product is derived from the causative organism and not from an irrelevant bacterial contaminant. The association of C. granulomatis with the Klebsiella species would have diagnostic as well as taxonomic implications. For example, donovanosis-specific PCR could be developed by use of primers targeting a less-conserved Klebsiella sequence. These improved diagnostic methods would assist in controlling donovanosis. Such interventions are urgently required in populations where donovanosis is endemic because, like other genital ulcerative diseases, donovanosis may increase the transmission of HIV [9, 10].

Ivan Bastian and Francis J. Bowden
Menzies School of Health Research, Casuarina, Darwin, Northern Territory, and AIDS/STD Unit, Disease Control Centre, Territory Health Services, Casuarina, Northern Territory, Australia

References
Native Valve Endocarditis Due to Corynebacterium striatum: First Reported Case of Medical Treatment Alone

The first case of native valve endocarditis due to Corynebacterium striatum required a combination of medical and surgical treatments for cure [1]. We describe the first patient with native valve endocarditis due to C. striatum who received only medical treatment.

A 24-year-old man was admitted to the hospital because of a persistent unexplained fever. His medical history was remarkable for congenital hydrocephalus that led to complete paraplegia and required an ventriculoperitoneal shunt at the age of 2 months. The shunt catheter was replaced when he was 16 years old because the distal extremity had migrated into the pulmonary artery. He had had an isolated fever 7 weeks before the current admission.

Providencia stuartii, C. striatum, and Escherichia coli were successively isolated from cultures of urine. The patient received therapy with ceftriaxone and cefixime for 1 week each; his fever resolved with therapy, but it returned as soon as the antibiotics were discontinued. A chest radiograph obtained 8 days before admission revealed a localized alveolar infiltrate in the lower lobe of the left lung. On admission, the alveolar infiltrate was not apparent.

C. striatum was isolated in three sets of blood cultures. A trans-thoracic echocardiogram revealed a 10-mm vegetation on the pulmonary valve that was close to the distal extremity of the ventriculoatrial shunt catheter and that was fluttering in the pulmonary artery. A transesophageal echocardiogram confirmed the pulmonary valve vegetation and did not reveal vegetation on the catheter.

The MICs and MBCs of amoxicillin, ceftriaxone, and netilmicin were 1 and 128 μg/mL, 8 and 64 μg/mL, and 0.03 and 0.03 μg/mL, respectively. The MICs of vancomycin and teicoplanin were 0.25 μg/mL and 0.25 μg/mL, respectively, and the MBCs of these drugs were 2 μg/mL and 0.25 μg/mL, respectively. The patient’s initial treatment included amoxicillin and netilmicin. Therapy with netilmicin was discontinued after 2 weeks. A sacral bedsores, which was considered the portal of entry of C. striatum, was treated surgically.

Six weeks after the treatment was started, while the patient was still receiving iv amoxicillin, he developed a sudden fever, chills, thoracic pain, and dyspnea. A localized alveolar infiltrate in the lower segment of the right lung was noted on a chest radiograph.

Three sets of blood cultures remained sterile. A transthoracic echocardiogram showed the size of the vegetation had not changed despite 7 weeks of therapy. Antibiotic treatment was then changed, in accordance with in vitro synergy studies (figure 1), to include amoxicillin, netilmicin, and iv teicoplanin. An endovascular procedure was attempted in order to remove the catheter in the pulmonary artery, but this procedure was unsuccessful. However, medical treatment led to improvement in the patient’s condition as the size of the vegetation decreased (seen on an echocardiogram).

After 4 weeks of therapy with amoxicillin, netilmicin, and teicoplanin, the patient was discharged from the hospital and continued to received therapy with oral amoxicillin for 2 more weeks. Antibiotic treatment was then discontinued; three sets of blood cultures remained sterile. No fever was noted at a follow-up visit 10 months later.

Twenty months after the initial episode, the patient was readmitted to our hospital because of fever. Clinical examination was unremarkable, and findings on a chest radiograph were normal. A

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Reprints or correspondence: Dr. Anne-Claude Crémeux, Hôpital Bichat-Claude Bernard, 46 Rue Henri-Huchard, 75877 Paris Cedex 18, France.

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Figure 1. The effect of antibiotics alone or in combination on the Corynebacterium striatum strain isolated from a patient with native valve endocarditis as determined on the basis of bactericidal kinetics. ▲ = control; ■ = amoxicillin (1 mg/L); ○ = teicoplanin (0.25 mg/L); □ = netilmicin (0.06 mg/L); ● = teicoplanin (0.25 mg/L) + netilmicin (0.06 mg/L); △ = amoxicillin (1 mg/L) + teicoplanin (0.25 mg/L); △ = amoxicillin (1 mg/L) + netilmicin (0.06 mg/L). The plots for ▲, ●, and △ are superimposed.