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CASE REPORTS

"*Mycobacterium canettii*" Isolated from a Human Immunodeficiency Virus-Positive Patient: First Case Recognized in the United States▽

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We report the first case of tuberculosis caused by "*Mycobacterium canettii*" recognized in the United States. The pathogen was isolated from the cerebrospinal fluid of a 30-year-old Sudanese refugee.

CASE REPORT

A 30-year-old female Sudanese refugee was admitted to a Baltimore, MD, hospital in January 2005 with advanced AIDS complicated by prior episodes of pneumonia and a reported history of treated tuberculosis. Two months earlier, the patient had been admitted to a Kampala, Uganda, hospital for treatment of severe wasting (weight upon admission was 35 kg). There she demonstrated a stiff neck, but no cells or microorganisms were detected in the cerebrospinal fluid (CSF), and "she recovered very quickly on fluids, food, and fluconazole." Three sputa were smear negative for acid-fast bacilli (AFB), and a chest radiograph was normal.

Upon admission to the Sinai Hospital of Baltimore, the patient complained of anorexia, nausea and vomiting, weight loss, right upper quadrant abdominal pain, inability to ambulate, and left foot pain. She was under an antiretroviral therapy that included stavudine, lamivudine, nevirapine, and sulfamethoxazole-trimethoprim for opportunistic infections. Her vital signs were normal but she appeared cachectic, with reduced muscle mass and strength, and had multiple hyperpigmented patches over both lower extremities, including a purplish discoloration on the plantar aspect of the left foot. Genital ulcers were present. The remainder of the examination was unremarkable.

Initial diagnostic studies revealed neutropenia with a white blood cell (WBC) count of 1,100 cells/mm^3 (64% polymorphonuclear cells, 16% lymphocytes, and 16% monocytes), profound cellular immunodeficiency with a CD4 count of 2 cells/mm^3, and a human immunodeficiency virus viral load of 2,040 copies/ml. Bacterial cultures of blood, urine, and sputum were negative for pathogens, and a chest radiograph was unremarkable. A punch biopsy of the purplish lesion of the left foot revealed Kaposi's sarcoma, and a helical computer-assisted tomography (CAT) scan of the abdomen and pelvis demonstrated scattered, hypodense lesions of both the liver and spleen thought to possibly represent either parenchymal Kaposi's sarcoma, disseminated fungal or mycobacterial process, or a pyogenic infection. A CAT scan of her head showed mild right cerebellar atrophy. Three blood cultures (Bactec 13A medium) were negative for mycobacteria, and a bone marrow biopsy revealed no granuloma and no organisms by Kinyoun's AFB staining, methenamine silver, or periodic acid-Schiff stains. Growth detection for mycobacteria of the bone marrow specimen was also negative in the Bactec 13A medium.

The patient was treated with ampicillin-sulbactam for possible hepatic microabscesses, acyclovir for genital herpes, and sulfamethoxazole-trimethoprim and weekly azithromycin for opportunistic infections. Anti-retroviral therapy was altered slightly to include abacavir, lamivudine, and nelfinavir. In spite of the applied treatment, the patient became progressively apathetic, with few verbal responses. A magnetic resonance imaging scan of the brain demonstrated a left parietal mass, abnormal enhancement of the medial frontal lobe, left frontal and anterior temporal lobe, and basal cisterns as well as the meninges. A CSF examination on the 21st hospital day revealed 89 WBC/mm^3 (82% polymorphonuclear cells, 16% lymphocytes, and 2% monocytes), 53 mg/ml glucose, and 209 mg/ml protein, and no organisms were seen by either Gram or Kinyoun staining. The CSF was negative for both the cryptococcal antigen test and a PCR assay for herpes simplex virus. Growth detection of the CSF was negative for mycobacteria, and a serum toxoplasmosis immunoglobulin G titer was also negative. Cytomegalovirus encephalitis was suspected, and ganciclovir was added to the treatment regimen. An oncology

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consultant suggested the possibility of disseminated Kaposi's sarcoma or non-Hodgkin lymphoma, the latter supported by the presence of Epstein-Barr virus DNA in CSF as determined by PCR (5,135 copies per ml).

In spite of the applied treatment, the patient's condition deteriorated, leading to progressive respiratory insufficiency which required intubation. Repeat CAT scans of the abdomen showed improvement in the hepatosplenic lesions. Cultures of blood and bone marrow showed no growth of AFB at 3 weeks of incubation on Bactec 13A medium. The patient developed generalized tonic-clonic seizures, and phenytoin was added. A repeated CSF examination (9 days after the first, on the 30th hospital day) revealed 873 WBC/mm^3 (92% polymorphonuclear cells), 70 mg/ml glucose, and 556 mg/ml protein, but still no organisms were seen by Gram and Kinyoun staining. Progressive and irreversible respiratory failure ensued such that the patient's family instructed that no further therapy be given. The patient fulfilled neurologic criteria for brain death on the 34th hospital day. No autopsy was performed.

All cultures (bone marrow, blood, and CSF from the 21st hospital day) were negative at 6 weeks for AFB, but the CSF from the 30th hospital day was reported to be growing an AFB in liquid medium approximately 2 weeks after the patient's demise.

The acid-fast organism that was isolated from the patient was identified as part of the *Mycobacterium tuberculosis* complex (MTC) by the AccuProbe TB assay (GenProbe Inc., San Diego, CA). On solid medium, the isolated MTC strain showed an eugonic, smooth, white, glossy colony morphology on Löwenstein-Jensen and Middlebrook 7H11 agar (Fig. 1). The strain was negative for niacin production and nitrate reduction and did not exhibit catalase activity after heat inactivation at 68°C. It was susceptible to first-line drugs (0.4 μg/ml isoniazid, 2.0 μg/ml rifampin, 2.5 and 7.5 μg/ml ethambutol, 2 and 6 μg/ml streptomycin; Bactec 460TB system) and was resistant to pyrazinamide (PZA; 100 μg/ml; Bactec 460TB system) and thiophene-2-carboxylic acid hydrazide (1, 5, and 10 μg/ml; agar proportion method) (11, 13). The unusual morphology and biochemical characterization prompted the referral of the isolate to a reference center for further identification to the species level.

Genetic analysis of the isolate was performed according to the following technologies: PCR-restriction fragment length polymorphism analysis of the *hsp65* gene, mutation analysis of the *pncA* gene by automated DNA sequencing, PCR-based genomic deletion analysis (region of difference 1 [RD1], RD4, RD9, RD10, RD12, and *M. tuberculosis*-specific deletion 1), and spoligotyping (8, 16, 17, 21, 22). Results of these assays revealed that the isolate carried the "*Mycobacterium canettii*"-specific C-to-T mutation in the *hsp65* gene (8) and the A-to-G mutation at position 138 in the *pncA* gene (22). Deletion analysis identified the presence of RD1, RD4, RD9, RD10, and tuberculosis-specific deletion 1 as well as the absence of RD12, also characteristic for "*M. canettii*" (10). Repeated spoligotyping indicated that all 43 spacer sequences were absent. On the basis of these phenotypic and genetic characteristics, the strain was identified as "*M. canettii*."

FIG. 1. Colony morphology of the "*Mycobacterium canettii*" isolate on Middlebrook 7H10 agar (4 weeks old).

The MTC consists of the closely related organisms *M. tuberculosis*, *M. africanum*, *M. bovis*, *M. bovis* BCG, *M. caprae*, *M. microti*, *M. pinnipedii*, the dassie bacillus, and "*M. canettii*" (2–4, 23). "*Mycobacterium canettii*" is a rarely identified member of the MTC that, according to our present knowledge, infects only humans and shows a geographical restriction to Africa (6, 18, 24).

"*M. canettii*" is characterized by eugonic growth, with smooth, white, glossy colonies on solid medium. Its biochemical characteristics indicate that it is unable to produce niacin, but it is capable of nitrate reduction and has positive urease, Tween 80 hydrolysis, and catalase activities (at 22°C, although not after heat inactivation at 68°C). It is naturally resistant to thiophene-2-carboxylic acid hydrazide and PZA (8, 18, 24). Genetically, it can be distinguished from other members of the MTC by an unusual composition of the direct repeat cluster when assessed by spoligotyping, by specific mutations in the *gyrB*, *hsp65*, and *pncA* genes, and by genomic deletion analysis (7–10, 22, 24).

To our knowledge, this is the first case in which tuberculosis attributable to "*M. canettii*" was recognized in the United States. Recognition and referral of unusual members of the MTC for further speciation is essential to effectively guide public health and primary care decisions because of the different epidemiology, host spectrum, geographic range, pathogenicity, and drug susceptibility of each member (2, 13, 16, 25). The accurate molecular identification of species of the MTC may help to guide public health and primary care decisions more effectively since (i) the level and target (humans and animals) of contact tracing can be different in cases of *M. tuberculosis* and *M. bovis* or *M. caprae* infections; (ii) unnecessary PZA treatment can be rapidly excluded from the regimen in the case of *M. bovis*, *M. bovis* BCG, and "*M. canettii*" infection (these members of the complex are naturally resistant to PZA) (8, 20); and (iii) the epidemiology and clinical significance of unusual members (i.e., *M. bovis*, "*M. canettii*," or *M. caprae*) of the complex in areas where they are more common but not identified can be better understood (5, 14, 15, 19).

Recent genetic studies suggest that "*M. canettii*" represents

one of the most ancient phylogenetic lineages of the tubercle bacilli (9, 12). The natural reservoir, host range, and mode of transmission of "*M. canettii*" are not adequately known since many clinical laboratories use tests that identify only to the MTC level. Therefore, the geographic distribution and prevalence of "*M. canettii*" are probably underestimated.

This report indicates that the rapid, accurate, and routine identification of "*M. canettii*" is useful to gain more information about its virulence, pathogenesis, and clinical presentation (i.e., pulmonary and extrapulmonary manifestation) and to better understand the clinical importance of this pathogen. Foreign-born patients account for an increased proportion of tuberculosis cases in the United States, which raises the importance of recognizing and rapidly differentiating the unusual members of the MTC (1).

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REFERENCES

1. **Anonymous.** 2008. Trends in tuberculosis–United States, 2007. MMWR Morb. Mortal. Wkly. Rep. **57:**281–285.
2. **Brosch, R., S. V. Gordon, M. Marmiesse, P. Brodin, C. Buchrieser, K. Eiglmeier, T. Garnier, C. Gutierrez, G. Hewinson, K. Kremer, L. M. Parsons, A. S. Pym, S. Samper, D. van Soolingen, and S. T. Cole.** 2002. A new evolutionary scenario for the *Mycobacterium tuberculosis* complex. Proc. Natl. Acad. Sci. USA **99:**3684–3689.
3. **Cousins, D. V., R. Bastida, A. Cataldi, V. Quse, S. Redrobe, S. Dow, P. Duignan, A. Murray, C. Dupont, N. Ahmed, D. M. Collins, W. R. Butler, D. Dawson, D. Rodriguez, J. Loureiro, M. I. Romano, A. Alito, M. Zumarraga, and A. Bernardelli.** 2003. Tuberculosis in seals caused by a novel member of the *Mycobacterium tuberculosis* complex: *Mycobacterium pinnipedii* sp. nov. Int. J. Syst. Evol. Microbiol. **53:**1305–1314.
4. **Cousins, D. V., R. L. Peet, W. T. Gaynor, S. N. Williams, and B. L. Gow.** 1994. Tuberculosis in imported hyrax (*Procavia capensis*) caused by an unusual variant belonging to the *Mycobacterium tuberculosis* complex. Vet. Microbiol. **42:**135–145.
5. **Dankner, W. M., and C. E. Davis.** 2000. *Mycobacterium bovis* as a significant cause of tuberculosis in children residing along the United States-Mexico border in the Baja California region. Pediatrics **105:**E79.
6. **Fabre, M., J.-L. Koeck, P. Le Flèche, F. Simon, V. Herve, G. Vergnaud, and C. Pourcel.** 2004. High genetic diversity revealed by variable-number tandem repeat genotyping and analysis of *hsp65* gene polymorphism in a large collection of "*Mycobacterium canettii*" strains indicates that the *M. tuberculosis* complex is a recently emerged clone of "*M. canettii*." J. Clin. Microbiol. **42:**3248–3255.
7. **Goh, K. S., M. Fabre, R. C. Huard, S. Schmid, C. Sola, and N. Rastogi.** 2006. Study of the *gyrB* gene polymorphism as a tool to differentiate among *Mycobacterium tuberculosis* complex subspecies further underlines the older evolutionary age of "*Mycobacterium canettii*." Mol. Cell. Probes **20:**182–190.
8. **Goh, K. S., E. Legrand, C. Sola, and N. Rastogi.** 2001. Rapid differentiation of "*Mycobacterium canettii*" from other *Mycobacterium tuberculosis* complex organisms by PCR-restriction analysis of the *hsp65* gene. J. Clin. Microbiol. **39:**3705–3708.
9. **Gutierrez, M. C., S. Brisse, R. Brosch, M. Fabre, B. Omais, M. Marmiesse, P. Supply, and V. Vincent.** 2005. Ancient origin and gene mosaicism of the progenitor of *Mycobacterium tuberculosis*. PLoS Pathog. **1:**e5.
10. **Huard, R. C., L. C. Lazzarini, W. R. Butler, D. van Soolingen, and J. L. Ho.** 2003. PCR-based method to differentiate the subspecies of the *Mycobacterium tuberculosis* complex on the basis of genomic deletions. J. Clin. Microbiol. **41:**1637–1650.
11. **Inderlied, C. B., and M. Salfinger.** 1999. Antimycobacterial agents and susceptibility tests, p. 1601–1623. *In* P. R. Murray, E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken (ed.), Manual of clinical microbiology, 7th ed. American Society for Microbiology, Washington, DC.
12. **Karboul, A., N. C. Gey van Pittius, A. Namouchi, V. Vincent, C. Sola, N. Rastogi, P. Suffys, M. Fabre, A. Cataldi, R. C. Huard, N. Kurepina, B. Kreiswirth, J. L. Ho, M. C. Gutierrez, and H. Mardassi.** 2006. Insights into the evolutionary history of tubercle bacilli as disclosed by genetic rearrangements within a PE_PGRS duplicated gene pair. BMC Evol. Biol. **6:**107.
13. **Kent, P. T., and G. P. Kubica.** 1985. Public health mycobacteriology. A guide for a level III laboratory. Centers for Disease Control, Atlanta, GA.
14. **LoBue, P. A., W. Betacourt, C. Peter, and K. S. Moser.** 2003. Epidemiology of *Mycobacterium bovis* disease in San Diego County, 1994–2000. Int. J. Tuberc. Lung Dis. **7:**180–185.
15. **Miltgen, J., M. Morillon, J. L. Koeck, A. Varnerot, J. F. Briant, G. Nguyen, D. Verrot, D. Bonnet, and V. Vincent.** 2002. Two cases of pulmonary tuberculosis caused by *Mycobacterium tuberculosis* subsp. *canetti.* Emerg. Infect. Dis. **8:**1350–1352.
16. **Parsons, L. M., R. Brosch, S. T. Cole, A. Somoskovi, A. Loder, G. Bretzel, D. Van Soolingen, Y. M. Hale, and M. Salfinger.** 2002. Rapid and simple approach for identification of *Mycobacterium tuberculosis* complex isolates by PCR-based genomic deletion analysis. J. Clin. Microbiol. **40:**2339–2345.
17. **Parsons, L. M., J. Dormandy, A. Clobridge, J. R. Driscoll, M. Oxtoby, H. W. Taber, and M. Salfinger.** 2004. Deletion analysis: a novel and rapid genotyping method for speciation within the *Mycobacterium tuberculosis* complex. Abstr. Nat. TB Controllers Workshop. National TB Controllers Association and CDC, Atlanta, GA.
18. **Pfyffer, G. E., R. Auckenthaler, J. D. van Embden, and D. van Soolingen.** 1998. *Mycobacterium canettii*, the smooth variant of *M. tuberculosis*, isolated from a Swiss patient exposed in Africa. Emerg. Infect. Dis. **4:**631–634.
19. **Prodinger, W. M., A. Eigentler, F. Allerberger, M. Schonbauer, and W. Glawischnig.** 2002. Infection of red deer, cattle, and humans with *Mycobacterium bovis* subsp. *caprae* in western Austria. J. Clin. Microbiol. **40:**2270–2272.
20. **Scorpio, A., D. Collins, D. Whipple, D. Cave, J. Bates, and Y. Zhang.** 1997. Rapid differentiation of bovine and human tubercle bacilli based on a characteristic mutation in the bovine pyrazinamidase gene. J. Clin. Microbiol. **35:**106–110.
21. **Scorpio, A., and Y. Zhang.** 1996. Mutations in *pncA*, a gene encoding pyrazinamidase/nicotinamidase, cause resistance to the antituberculous drug pyrazinamide in tubercle bacillus. Nat. Med. **2:**662–667.
22. **Somoskovi, A., J. Dormandy, L. M. Parsons, M. Kaswa, K. S. Goh, N. Rastogi, and M. Salfinger.** 2007. Sequencing of the *pncA* gene in members of the *Mycobacterium tuberculosis* complex has important diagnostic applications: identification of a species-specific *pncA* mutation in "*Mycobacterium canettii*" and the reliable and rapid predictor of pyrazinamide resistance. J. Clin. Microbiol. **45:**595–599.
23. **Tsukamura, M., S. Mizuno, and H. Toyama.** 1985. Taxonomic studies on the *Mycobacterium tuberculosis* series. Microbiol. Immunol. **29:**285–299.
24. **van Soolingen, D., T. Hoogenboezem, P. E. de Haas, P. W. Hermans, M. A. Koedam, K. S. Teppema, P. J. Brennan, G. S. Besra, F. Portaels, J. Top, L. M. Schouls, and J. D. van Embden.** 1997. A novel pathogenic taxon of the *Mycobacterium tuberculosis* complex, Canetti: characterization of an exceptional isolate from Africa. Int. J. Syst. Bacteriol. **47:**1236–1245.
25. **Wayne, L. G., and G. P. Kubica.** 1986. The mycobacteria, p. 1435–1457. *In* P. H. A. Sneath and J. G. Holt (ed.), Bergey's manual of systemic bacteriology, vol. 2. The Williams and Wilkins Co., Baltimore, MD.