

## Lethal *Mycobacterium massiliense* Sepsis, Italy

**To the Editor:** A strain of *Mycobacterium massiliense* was isolated from the blood of a kidney transplant patient in Italy at the same time she was diagnosed as having pulmonary tuberculosis. *M. massiliense* bacteremia appears to have played a role in her sudden death.

The patient, a 63-year-old woman who had had a kidney transplant 10 years earlier and was receiving immunosuppressive treatment with cyclosporine, azathioprine, and prednisolone, was hospitalized in an intensive care unit because of septic shock, a stuporous condition, hypotension, respiratory insufficiency, and acute renal failure. Results of initial microbiologic investigations (cultures of blood, urine, and bronchial aspirate, and nasal and pharyngeal swabs; tests for antigens of *Legionella pneumophila*, pneumococcus, and *Cryptococcus neoformans*; and serologic analysis for pneumotropic viruses and bacteria) were negative.

Hematologic analysis showed leukopenia with high neutrophil counts. Septic shock-specific therapy was given along with wide-spectrum antimicrobial drug therapy (levofloxacin, trimethoprim-sulfamethoxazole, piperacillin-tazobactam, and fluconazole). After moderate improvement, the patient's general condition worsened and prompted a new round of microbiologic tests, including those for mycobacteria. After acid-fast bacilli were detected in a bronchial aspirate and results of nucleic acid amplification (Amplicor; Roche, Basel, Switzerland) were positive for *M. tuberculosis* complex, she was given standard antituberculosis treatment. She died the next day.

Three blood cultures obtained 7 days before the death of the patient

showed positive results in aerobic bottles but not in anaerobic bottles (BacT/ALERT SA; bioMérieux, Marcy l'Etoile, France). Subcultures spread onto blood agar yielded small white colonies of gram-positive bacilli, including branched forms, within 24 hours. One month earlier, the patient had been seen as an outpatient with intermittent fever, and unidentified gram-positive bacilli were observed in her blood culture.

Genetic sequencing of the first one third of the 16S rDNA gene (1) of the strain (GenBank accession no. EU370523) showed 99.8% identity with *M. abscessus*, *M. bolletii*, *M. chelonae*, and *M. massiliense*. To discriminate between these 4 species, a 723-bp fragment of the RNA polymerase  $\beta$  subunit (*rpoB*) gene (2) also was sequenced (GenBank accession no. EU370524). Sequencing showed 100% similarity with *M. massiliense*; the next most closely related species was *M. bolletii* (98.6% similarity).

An isolate of *M. tuberculosis* that was susceptible to all first-line antituberculosis drugs was recovered from this patient's bronchial aspirate in both solid and liquid media. Immunocompromised patients, including those with organ transplants, are known to be prone to mycobacterial infections (3). Mixed mycobacterial infections also have been reported (4). This newly described species has been isolated from pulmonary fluids (5), blood (6), intramuscular injection sites

(7), and surgical wounds (8). Because sequencing 16S rDNA does not differentiate *M. abscessus*, *M. bolletii*, *M. chelonae*, and *M. massiliense* (5), use of the *rpoB* sequence is crucial.

As reported for isolates of *M. massiliense* (5–8), our isolate was characterized by high MICs (9) (broth microdilution by using the Sensititer RGMCO; Trek Diagnostic Systems Inc., Cleveland, OH, USA) to most of the antimicrobial drugs tested. The isolate was sensitive only to clarithromycin and amikacin and showed borderline sensitivity to linezolid (Table).

Despite co-infection with *M. tuberculosis*, the patient's death was likely caused by *M. massiliense* bacteremia. The patient died before the isolate was identified as a mycobacterium, and unfortunately, none of the drugs used empirically was active against this organism.

Only speculations can be made about how this patient acquired the *M. massiliense* infection. However, 5 months before her hospital admission, the patient had received a coxofemoral arthroprosthesis as a result of a fall and had since complained of generalized bone pain and had remained bedridden. Although in this case no proof exists, infection caused by rapidly growing mycobacteria after surgical intervention (10) is well known and should be considered.

*M. massiliense* has been distinguished from *M. abscessus* by sequencing of the *rpoB* gene (2). Be-

Table. Antimicrobial drug resistance pattern of the strain of *Mycobacterium massiliense* isolated from the patient, Italy\*

Drug	MIC ( $\mu\text{g/mL}$ )	Pattern
Amikacin	4	S
Amoxicillin/clavulanic acid	64/32	R
Cefoxitin	64	I
Ceftriaxone	>64	R
Ciprofloxacin	16	R
Clarithromycin	$\leq 0.12$	S
Gatifloxacin	>8	R
Imipenem	32	R
Linezolid	8	S
Tobramycin	16	R
Trimethoprim/sulfamethoxazole	8/152	R

\*S, sensitive; R, resistant; I, intermediate resistance.

cause this technology is available in relatively few clinical laboratories, cases of infection with *M. massiliense* may be mistakenly attributed to *M. abscessus*. Although infections with *M. massiliense* may be underrecognized, reports of these infections are raising concern. The capacity of this bacteria to infect different body sites is further evidence for the pathogenic potential of a rapidly growing mycobacteria in human infections (10).

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## Bovine Kobuviruses from Cattle with Diarrhea

**To the Editor:** A new species of kobuvirus, named U-1 strain, was first recognized in 2003 as a cytopathic contaminant in a culture medium of HeLa cells that had been used for >30 years in the laboratory (1). The RNA genome of the U-1 strain comprises 8,374 nucleotides; the genome organization is analogous to that of picornaviruses. Morphologically, the U-1 strain resembles the Aichi virus, but genetically it is distinct (1). Therefore, the U-1 strain is classified as a new species of genus *Kobuvirus* in the family *Picornaviridae*, and it is called *bovine kobuvirus* (1). To date, the genus *Kobuvirus* consists of 2 species, *Aichi virus* and *bovine kobuvirus* (2). The Aichi virus is associated with acute gastroenteritis in humans (3–5); bovine kobuvirus infection has been detected only in cattle (1).

Only 1 report has described the discovery and epidemiologic features

of bovine kobuvirus (1). Of serum samples from 72 healthy cattle, 43 (59.7%) were positive for neutralizing antibody against bovine kobuvirus U-1 standard strain at a titer of  $\geq 16$ . In addition, 12 (16.7%) of 72 stool samples collected from the cattle were positive for the bovine kobuvirus genome by reverse transcription-PCR (RT-PCR) (1). This finding suggested that bovine kobuvirus is common and that the virus particles could be detected in the stool samples of infected cattle. We therefore conducted an epidemiologic survey of bovine kobuvirus and report detection of this virus in stool samples from calves with diarrhea during 2001–2004 in Chiang Mai Province, Thailand.

From November 2001 to July 2004, a total of 72 fecal specimens were collected. The age of the calves ranged from 7 to 49 days. The presence of bovine kobuvirus in fecal specimens was detected by using RT-PCR with a protocol modified from the method described by Yamashita et al. (1). All the bovine kobuvirus strains detected in our study were analyzed further by direct sequencing of their PCR amplicons with the BigDye Terminator Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA) on an automated sequencer (ABI 3100, Applied Biosystems). The nucleotide sequences of these portions were compared with those of reference strains available in the GenBank database by using BLAST (6). Phylogenetic and molecular evolutionary analyses were conducted by using MEGA version 3.1 (7). The nucleotide sequences of bovine kobuvirus strains described in this study were deposited in GenBank under accession nos. EF659450–EF659455.

The bovine kobuvirus was detected by the RT-PCR screening method in 6 (8.3%) of the 72 fecal specimens collected. The partial 3D regions of all 6 bovine kobuviruses exhibited highly conserved sequences of 99.3%–100% nucleotide and 100% amino acid