Table. Factors associated with isoniazid resistance among *Mycobacterium tuberculosis* isolates, National Taiwan University Hospital, Taiwan, 2000–2010*

Factor	No. infections	No. isoniazid-resistant infections	Resistance rate	OR (95% CI)
Patient age, y				
<14	42	2	4.76	0.32 (0.08–1.23)
14 to <24	241	22	9.13	0.64 (0.42–1.07)
24 to <34	342	36	10.53	0.75 (0.48–1.18)
34 to <44	384	52	13.54	Reference
44 to <54	490	56	11.43	0.82 (0.55–1.23)
54 to <64	609	70	11.49	0.83 (0.57–1.22)
64 to <74	845	96	11.36	0.82 (0.57–1.17)
74 to <84	986	85	8.62	0.60 (0.42–0.87)
84	350	28	8.00	0.56 (0.34–0.90)
Patient sex				
F	1,356	128	9.44	0.85 (0.69–1.06)
M	2,933	319	10.88	Reference
Pulmonary tuberculosis				
No	772	56	7.25	0.63 (0.47–0.84)
Yes	3,517	391	11.12	Reference

*OR, odds ratio; CI, confidence interval.

receive isoniazid if their TB developed when they were young. In the present study, the resistant rate was lower for *M. tuberculosis* strains isolated from elderly persons than from younger adults. These findings suggest that first-line anti-TB medications still have good in vitro activity against *M. tuberculosis* strains in elderly patients.

In contrast to the study by Vinnard et al. (*1*), our results showed that isoniazid-resistant *M. tuberculosis* was significantly less likely to be isolated from nonrespiratory than from respiratory specimens. The reasons for this finding are unclear. Continuous monitoring of antimicrobial drug resistance among *M. tuberculosis* isolates isolated from various body sites needs to be incorporated into any TB surveillance program.

Gathering data on drug resistance rates is a major aspect of the global TB control program. Clinicians must have knowledge of local epidemiology, and mycobacteriology laboratories should maintain up-to-date information on drug susceptibility test profiles of local *M. tuberculosis* isolates.

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Novel *Mycobacterium* Species in Seahorses with Tail Rot

To the Editor: Seahorses (*Hippocampus guttulatus* and *H. hippocampus*) with signs of tail rot disease (lethargy, lack of appetite, white spots on the skin, and necrotic tail lesions) were collected from aquaria at the Institute of Marine Research, Spain, during March 2007 through May 2009 (online Appendix Figure, www.cdc.gov/EID/content/17/9/101289-appF.htm). Microscopic examination of cutaneous lesions after Ziehl-Neelsen staining disclosed acid-fast bacilli. Microbiologic analysis showed unidentified *Mycobacterium* strains. Subsequently, we used PCR amplification of repetitive bacterial DNA elements to group the strains (*1*). The results showed an identical PCR pattern for the strains; thus, we selected strain BFLP-6^T for analysis. On the basis of phenotypic and genotypic data, we consider the unknown acid-fast bacillus to represent a novel species of the genus *Mycobacterium*, for which the name *M. hippocampi* sp. nov. is proposed.

Extraction and amplification of genomic DNA for 16S rRNA sequence analysis were conducted as described (*2*), and the RNA polymerase B (*rpoB*) gene was amplified and sequenced as described by Adékambi et al. (*3*).

Sequences obtained were compared against the sequences available in the GenBank, EMBL, and DDBJ databases obtained from the National Center for Biotechnology Information by using the BLAST program (*4*). Phylogenetic analysis were performed by using MEGA version 4.0 (*5*) after multiple alignments of data by ClustalX (*6*). Distances (distance options according to the Kimura 2-parameter model) and clustering with the neighbor-joining method were determined by using bootstrap values for 1,000 replications.

The 16S rRNA sequence of strain BFLP-6^T was a continuous stretch of 1,473 bp (GenBank accession no. FN430736). Sequence similarity calculations after a neighbor-joining analysis indicated that the closest relatives of strain BFLP-6^T were *M. flavescens* (98.26%), *M. goodii* (98.01%), *M. duvalii* (97.94%), *M. smegmatis* (97.92%), and *M. novocastrense* (97.86%) (Figure). Similar results were obtained for strain BFLP-6^T when the maximum-parsimony algorithm was used. The *rpoB* gene has also been proposed as a useful marker for inferring bacterial phylogeny (*7*,*8*). A pairwise analysis of the *rpoB* sequence of strain BFLP-6^T (GenBank accession no. FR775976) showed low levels of similarity (<89.8%) with other species of the genus *Mycobacterium*. The G + C content of DNA, as measured by the thermal denaturation method, was 66.7 mol%.

Strain BFLP-6^T was found to consist of gram-positive–staining, aerobic, acid-alcohol–fast, nonmotile, and nonsporulating cells. A scanning electron micrograph showed that strain BFLP-6^T is irregular, rod-shaped, ≈1.2–1.4 μm in length, and 0.4 μm in diameter. Colonies on Lowenstein-Jensen medium supplemented with 1.5% (wt/vol) sodium chloride were orange after incubation at 25°C for 5 days. The colonies were positive for catalase, glucose fermentation, arginine dihydrolase, urease, and aesculin, and assimilation of glucose, mannitol, potassium gluconate, and malate. The colonies were negative for nitrate reduction to nitrite, oxidase, indole production, gelatin hydrolysis, *N*-acetyl-D-glucosamine; and assimilation of arabinose, mannose, maltose, caprate, adipate, citrate, and phenylacetate. The major fatty acids were C18:1ω9*c*, C16:0, and C16:1ω6*c*. Mycolic acids included α-mycolates, keto-mycolates, and nonhydroxylated fatty acid methyl esters.

In addition, strain BFLP-6^T showed resistance to isoniazid, thiophene-2-carboxylic hydrazide, hydroxylamine, thiacetazone, and picrate. However, the strain exhibited susceptibility to ciprofloxacin, clarithromycin, and rifampin. The type strain BFLP-6^T has been deposited in the German Collection of Microorganisms and Cell Cultures, under reference DSM 45391^T; and in the Belgian Coordinated Collections of Microorganisms under reference LMG 25372^T.

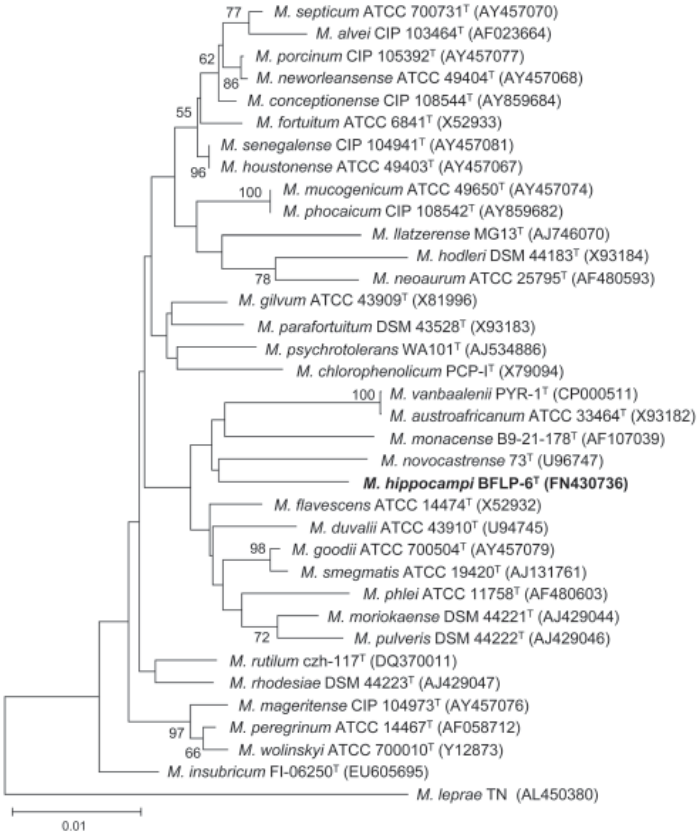

Figure. Neighbor-joining phylogenetic tree constructed from 16S rRNA gene sequences, showing the position of strain BFLP-6^T (in **boldface**) among other *Mycobacterium* species. Numbers at node indicate bootstrap values (expressed as percentages of 1,000 replications); only values >50% are given. *Mycobacterium leprae* TN was used as an outgroup. Scale bar indicates 0.01 substitutions per nucleotide position. GenBank accession numbers are in parentheses.

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Mycoplasma leachii sp. nov. in Calves, China

To the Editor: *Mycoplasma leachii* sp. nov., a new species designation for *Mycoplasma* sp. bovine group 7 (*1*), was initially isolated from joint fluids of arthritic calves in southern Queensland, Australia, and its pathogenicity was established by experimental infection (*2*). It was represented by the type strain PG50. Subsequently, *M. leachii* was reported infrequently as a cause of polyarthritis in calves and mastitis in cows; the pathogen was also isolated from aborted fetuses and pneumonic bovine lungs (*3*–*6*) and from small ruminant hosts (*7*).

M. leachii is one of 5 recognized members of the *M. mycoides* cluster, which comprises 3 species (*1*). Most notable are *M. mycoides* subsp. *mycoides* small colony and *M. capricolum* subsp. *capripneumoniae*, the etiologic agents of contagious bovine and caprine pleuropneumonia, which are listed by the World Organisation for Animal Health as notifiable animal diseases. The *M. mycoides* subsp. *capri* and *M. capricolum* subsp. *capricolum* cause various symptoms in small ruminants (*8*). Strains of *M. leachii* that cause mastitis and polyarthritis in cattle are serologically distinct from other bovine *Mycoplasma* spp. (*9*). Most reported isolates of *M. leachii* were detected in Australia. We report the isolation of *M. leachii* in cattle in China.

During January–May 2009, severe polyarthritis was observed in ≈100% of ≈350 female calves at the central calf rearing unit of a farm in Helongjiang Province, People's Republic of China. Clinical signs were noticed at ≈3–5 days of age, with severity gradually increasing over the next 2 days. At that time, the carpal and tarsal joints were greatly enlarged because of accumulation of intraarticular fluid. Ampicillin, sulfonamide, and streptomycin antimicrobial drug regimens for polyarthritis were ineffective. Approximately 100 calves died during the outbreak; the remaining calves recovered irrespective of treatment, but permanent disfigurement of the appendicular skeleton was evident. The disfigurement led to the calves being culled.

Necropsy was conducted on the calves that died during the outbreak, and gross and histopathologic findings similar to those described (*2*,*3*) were observed. Nearly all diarthroidal joints were enlarged and contained yellow-gray turbid synovial fluid and large yellow fibrin clots. The synovial membranes were slightly thickened, congested, and had some villous proliferation. Histologic examination of the affected articulations found severe, diffuse, subacute arthrosynovitis and bursitis.

Routine bacterial culture of 2 joint fluid samples collected aseptically from different animals showed no bacterial growth. *Mycoplasma* spp. infection was suspected, and the samples were forwarded to the laboratory for specific culture; 2 were positive for *Mycoplasma* spp. These isolates were designated GN407 and GN408.

The presence of *M. leachii* in joint fluids and *Mycoplasma* spp.–positive cultures was detected by PCR with the partial *lppA* gene amplified with a protocol modified from the method described by Frey et al. (*10*) and amplification of the complete 16S rRNA gene was performed by using the primers 16S-upper 5′-AAAATGAGAGTTTGATCCTGG-3′ and 16S-lower 5′-AGAAAGGAGGTGATCCATCCG-3′. The primers were designed on the basis of the 16S rRNA gene sequence of *M. leachii* PG50 (U26054). PCR products were sequenced directly in both directions. Sequence analyses