
Mycobacterium africanum Cases, California

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Five *Mycobacterium tuberculosis* complex isolates in California were identified as *M. africanum* by spoligotyping, single nucleotide polymorphisms, a deletion mutation, and phenotypic traits, confirming it as a cause of tuberculosis in the United States. Three of the five patients from whom *M. africanum* was isolated had lived in Africa.

Mycobacterium africanum is a member of the *M. tuberculosis* complex, which has been isolated from humans in equatorial Africa. The disease produced by *M. africanum* is similar to that caused by *M. tuberculosis* or *M. bovis*, and like *M. tuberculosis*, this organism is likely spread by aerosol transmission (1). Human tuberculosis caused by *M. africanum* has been reported in Europe (2,3). However, we are unaware of previous reports of disease caused by *M. africanum* in the United States.

The Study

M. africanum may be identified by spoligotyping (4), by specific deletion mutations (5), DNA fingerprinting by IS6110 restriction fragment length polymorphisms (RFLP) (4), or a combination of these methods. Isolates were initially identified as *M. tuberculosis* complex by using the AccuProbe system (Gen-Probe; San Diego, CA). The isolates then underwent IS6110-based RFLP fingerprinting. The RFLP analyses were performed according to the method of van Embden et al. (6). In addition to providing genotyping results, RFLP fingerprinting confirmed the identification obtained with AccuProbe.

All strain typing was performed in-house at the Microbial Diseases Laboratory, California Department of Health Services. This laboratory has compiled a database (Genomic Solutions BioImage) of approximately 7,000 DNA fingerprints, typed by IS6110 RFLP (6,7) from throughout California; most are from the San Francisco Bay area. Three isolates (from patients A, B, and C) were initially suspected of being *M. africanum* because of an epidemiologic association with Africa. These isolates were fingerprinted by IS6110 RFLP and by spoligotyping (8).

All three were found to have the “signature” spoligotype described by Viana-Niero et al. as being characteristic of *M. africanum* (4), namely, they were missing spacers 8, 9, and 39 but had spacers 40–43. Using BioImage software, we searched the laboratory’s database for IS6110 fingerprints that matched those of the three cases with an African connection. This search yielded an additional two matches, cases D and E. Isolates from cases D and E were then genotyped by using spoligotyping and found to have the *M. africanum* signature spoligotype.

The five *M. africanum* isolates were further characterized by performing standard biochemical identification tests and testing for susceptibility to pyrazinamide (PZA). Niacin production and nitrate reduction were detected as described by Kent and Kubica (9). Susceptibility to PZA was determined by using the BACTEC radiometric assay performed according to the method of Salfinger et al. (10). The *M. africanum* isolates were then examined to determine whether they had the RD9 deletion and specific *oxyR* and *katG* sequence mutations.

Brosch et al. had reported that isolates of *M. tuberculosis* do not have the RD9 deletion, whereas other members of the *M. tuberculosis* complex, including *M. bovis*, *M. microti*, and *M. africanum* have this deletion (5). Two sets of primers for detecting the RD9 deletion, designed as described by Brosch et al., were obtained from Alex Pym at Stanford University. A ≈480-bp region was amplified by polymerase chain reaction (PCR) from *M. africanum* by using the flanking primers, and a ≈375-bp region was amplified from *M. tuberculosis* by using the internal primers. After an initial denaturation step of 10 min at 95°C, amplification was performed for 35 cycles at 95°C for 30 s, 55°C for 1 min, and 72°C for 4 min, and a final step of elongation at 72°C for 10 min. The final concentration of each component in the 50-μL PCR reaction tube was 1x PCR buffer, 2.5 mmol/L of MgCl₂, and 1.5 U of AmpliTaq Gold polymerase using AmpliTaq Gold kit with GeneAmp 10x PCR buffer II and MgCl₂ solution (Applied Biosystems, Foster City, CA), 1.25 mmol/L of deoxynucleotide triphosphate mix (Applied Biosystems), 0.2 μmol/L of each primer, 5 μL of DNA template. A *M. tuberculosis* strain, H37Rv, and *M. africanum* strain ATCC 25420 were included in PCR runs as reference strains.

A 548-bp region of the *oxyR* locus was amplified by PCR using the primer sequences described by Sreevatsen et al. (11). After an initial denaturation step of 4 min at 95°C, amplification was performed for 30 cycles with the following parameters: 95°C for 30 s, 55°C for 30 s, and 72°C for 45 s. A final elongation step was performed for 5 min at 72°C. For *katG*, a primer set, 5'-TGCTG-GCGCTTGGCAATACA and 5'-GCCGCGCTTGTCGC-TACC, was designed to amplify a 429-bp region encompassing codon 463. With the exception of an anneal-

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ing temperature of 60°C, the amplification parameters for *katG* were identical to those described for *oxyR*. The amplified products were purified by using QIAquick spin columns (Qiagen Inc., Valencia, CA) and subjected to dRhodamine Terminator Cycle Sequencing (Applied Biosystems, Foster City, CA) as recommended by the manufacturer. The cycle sequencing reactions were analyzed on an ABI 377 DNA sequencer, and the sequences aligned with those derived from *M. tuberculosis* strain H37Rv and *M. africanum* strain ATCC 25420.

For case histories, see Table 1. The five isolates were identified as *M. tuberculosis* complex by a positive result with the Gen-Probe AccuProbe system and by the presence of IS6110 insertion sequences. When spoligotyping was performed on isolates from cases A through E, all were found to have the *M. africanum* signature spoligotype, as described by Viana-Niero et al. (4). The results of spoligotyping are shown in the Figure.

The five isolates were *M. africanum*, based on several criteria shown in Table 2. Susceptibility of the strains to PZA, nitrate reduction, production of niacin, and the presence of guanosine at *oxyR*285 were incompatible with identification of the isolates as *M. bovis*. The RD9 deletion was shown for all five isolates and the *M. africanum* reference strain, ATCC 25420, by 1) the presence of a ~480-bp band on the agarose gel when the PCR was performed with the flanking primers and 2) the absence of a ~375-bp band when the PCR was performed with the internal primers. The RD9 deletion was incompatible with identification of

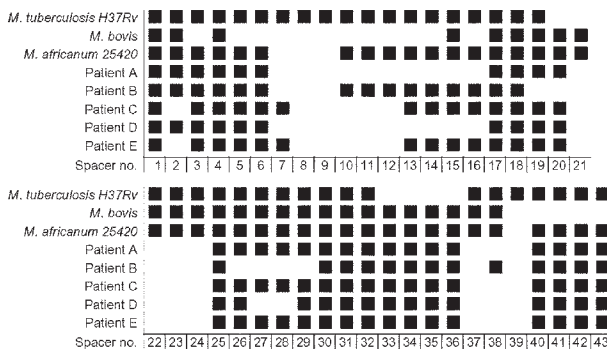


Figure. Spacer oligonucleotide typing (spoligotyping) results for cultures from patients A through E, together with representative patterns from stock cultures of *Mycobacterium tuberculosis* complex species. Spoligotypes for patients C and E are identical.

the isolates as *M. tuberculosis*, and CTG at *katG*463 suggested that the isolates were not *M. tuberculosis*, since most isolates of this species have CGG at this site (7).

Conclusions

David et al. (12) and Frothingham et al. (13) reported that some isolates of *M. africanum* are resistant to PZA and fail to accumulate niacin, distinguishing them from most strains of *M. tuberculosis*, while other isolates are susceptible to PZA and produce niacin. These five isolates of *M. africanum* were susceptible to PZA, and three out of the five tested positive for niacin production and nitrate reduction (the remaining two cultures had lost viability and were

Table 1. Clinical characteristics of patients with *Mycobacterium africanum* disease

Patient	Demographic characteristics	Signs and symptoms	Clinical characteristics	Treatment ^a	Outcome
A	25-y-old black South African man with recent travel to West Africa	3 months' productive cough, weight loss, and low-grade fever	Large left upper lobe infiltrate on chest radiograph	INH, rifampin, PZA, and ethambutol by directly observed therapy for 6 months	Resolution of symptoms and improvement of chest radiograph
B	34-y-old black West African woman recently arrived in U.S. with metastatic carcinoma, treated with chemotherapy 1 year before presentation.	2 months' cough, fever, night sweats, fatigue	Right lower lobe cavitation, streaky infiltrates bilaterally on chest radiograph, normocytic anemia	INH, rifampin, PZA, and ethambutol by DOT	Sputum culture negative after 2 weeks of therapy. Only modest improvement in chest radiograph. Returned to West Africa after 4 months DOT
C	26-y-old black South African, immigrated to U.S. 2 y before diagnosis	Unknown	Cavitary lesion on chest radiograph	INH, rifampin, PZA, and ethambutol by DOT for 6 months	Sputum culture and smear negative, chest radiograph improved
D	26-y-old Vietnamese man, immigrated to U.S. 6 y before diagnosis. No history of travel to Africa or African contacts with tuberculosis	2 weeks' productive cough, positive PPD	Unknown	Capreomycin, ofloxacin, PAS, clofazamine, and cycloserine for 2 y by DOT	Sputum smear and culture converted to negative, symptoms resolved. Free of disease 2 y after therapy completed
E	27-y-old U.S.-born black man with no known risk factors for tuberculosis infection. No history of travel to Africa or African contacts with tuberculosis	2 months' anorexia and weight loss; 1 month of cough and night sweats; 1 week of fever and abdominal pain	Normal chest radiograph results. Pancreatic cyst and fullness of left psoas muscle on CT scan	INH, rifampin, PZA, and ethambutol for 10 months by DOT	Symptoms resolved. Free of disease 1 y after therapy completed

^aINH, isoniazid; PZA, pyrazinamide; DOT, directly observed therapy; CT, computerized tomography; PPD, purified protein derivative; PAS, para-aminosalicylic acid.

Table 2. Phenotypic and molecular characteristics of *Mycobacterium africanum* isolates and country of birth for five patients

Patient	No. of copies of IS6110 (bands)	Spoligo-typing pattern missing spacers 8,9,39?	OxyR 285 ^a	Codon katG463 sequence ^b	Presence of RD9 deletion?	PZA susceptibility ^c	Niacin production	Nitrate reduction ^d	Major site of disease	Birth country
A	10	Yes	G	CTG	Yes	S	+	3+	Lung	South Africa
B	3	Yes	G	CTG	Yes	S	ND ^e	ND	Lung	Ivory Coast
C	9 ^f	Yes	G	CTG	Yes	S	ND	ND	Lung	South Africa
D	11	Yes	G	CTG	Yes	S	+	4+	Lung	Vietnam
E	9	Yes	G	CTG	Yes	S	+	3+	Pancreas	USA

^aIsolates of *M. bovis* do not have G at this site.

^bCTG is found in *M. bovis* and *M. africanum*, but not in most strains of *M. tuberculosis*.

^cS, susceptible; PZA, pyrazinamide.

^d3+ and 4+ reactions are considered positive.

^eND, not done (because the culture lost viability).

^fRestriction fragment length polymorphism patterns for isolates from patients C and E were identical.

not available for this testing). This rendered the cultures phenotypically indistinguishable from *M. tuberculosis* (12–14). For each of the reported cases, the signature *M. africanum* spoligotype pattern described by Viana-Niero et al. (4) was supported by the single nucleotide polymorphisms characteristic of *M. africanum* and by the RD9 mutation that characterizes members of the tuberculosis complex other than *M. tuberculosis*.

At present, the Microbial Diseases Laboratory plans to spoligotype all strains of *M. tuberculosis* complex. Clinical features and drug susceptibility were not distinctive for *M. africanum* isolates, nor were all associated with patients who came from Africa. No connection with a source case from Africa was found for patients D or E. However, since spoligotyping will be routine, noting the signature *africanum* spoligotype may be worthwhile. This spoligotype may indicate an increased chance that the patient may have acquired the *M. tuberculosis* complex infection in Africa or possibly from an African source case.

Dr. Desmond has worked at the Microbial Diseases Laboratory, California Department of Health Services, since 1990. His research interests include laboratory methods for detecting drug resistance in *Mycobacterium tuberculosis* and applications for molecular strain typing in the mycobacteriology laboratory.

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