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Mycobacterium yongonense in Pulmonary Disease, Italy

To the Editor: Mycobacterium yongonense is a recently described species (1) that belongs to the M. avium complex (MAC) and is associated with pulmonary infection. The strain on which the description of species was based was isolated in South Korea from the sputum of a patient with unspecified pulmonary disease. We describe 2 M. yongonense strains isolated from patients in Italy.

Patient 1 was a 74-year-old woman who had experienced fatigue, diarrhea, and weight loss. Her medical history included liver cirrhosis resulting from hepatitis C virus infection and surgery for colon cancer; the patient also reported tuberculosis in childhood. Chest radiograph revealed a cavitary lesion, a finding confirmed by computed tomography scan (Figure). Cultures in liquid and solid media grew a nonchromogenic mycobacterium from sputum and stool samples; results were negative for urine samples.

The patient was treated with clarithromycin, rifabutin, and ethambutol and showed some improvement. A bronchoscopic investigation was performed, and microscopic examination of bronchoalveolar lavage samples revealed the presence of acid-fast bacilli that subsequently were grown in culture. The patient began improving markedly starting with the second month of treatment, which will be continued for a total of 18 months.

Patient 2 was a 74-year-old woman, living in a community of nuns, who reported cough and dyspnea. Her medical history included renal failure and surgery for breast cancer. A bronchoalveolar lavage was performed; samples yielded in culture Pseudomonas aeruginosa and a nonchromogenic mycobacterium.
The patient was treated with ce-
fepime, to which \textit{P. aeruginosa} was
susceptible in vitro, and rapidly im-
proved. The isolation of the nontu-
berculous mycobacterium was con-
sidered irrelevant, and no specific
treatment was undertaken.

To determine the specific myco-
bacteria species isolated from these
patients, we conducted a commercial
line-probe assay (GenoType Myco-
bacterium CM; Hain Lifesciences,
Nehren, Germany). Both strains were
identified as \textit{M. intracellulare}. How-
ever, the known cross-reaction of
\textit{M. intracellulare} probe with most MAC
species \cite{2} led us to determine the
complete sequence of the 16S rRNA
gene. Both strains showed 100%
similarity with \textit{M. yongonense} and \textit{M. marseillense} \cite{3} strains.

To confirm this unusual finding,
we investigated other genetic
regions. We detected 100% identity
with \textit{M. yongonense} in the internal
transcribed spacer 1 region and in a
1,384-bp region of the \textit{hsp65} gene
and found 2 mismatches in a 420-bp
fragment of the \textit{sodA} gene (99.5%
similarity). In contrast, \textit{M. marsei-
llense} showed 6 mismatches (98.6%
similarity) in the internal transcribed
spacer 1 region and 24 (98.3% simi-
larity) in \textit{hsp65}; no \textit{sodA} sequence is
available in GenBank for this species.
Partial sequencing of other genetic
targets not available in GenBank for
\textit{M. yongonense} enabled us to confirm
the close relatedness of the strains to
\textit{M. intracellulare} (100% similarity in
d\textit{naK} gene; 99.3% identity in \textit{gyrB}
and \textit{gyrC} genes).

The finding of the same novel
\textit{Mycobacterium} species in these 2
unrelated patients reflects variability
in the significance of nontuberculous
mycobacteria isolated from clini-
cal specimens. \textit{M. yongonense} was
probably a contaminant in the second
case, but in the first, its involvement
as causative agent of disease seems
incontrovertible. The specific criteria
of the American Thoracic Society \cite{4}
were fulfilled: radiographic imaging
clearly documented the presence of
a cavitary pulmonary lesion, no other
pathogen possibly responsible of dis-
 ease was detected by bronchoscopic
investigation, and the same mycobac-
teria was isolated repeatedly from
sputum (its presence in stool prob-
able results from swallowed sputum)
and bronchoalveolar lavage samples.
Confirmation is further provided by
the response to the specific therapy,
according to international guidelines
\cite{4,5}, for MAC pulmonary disease
(MICs were 2, 1, and 8 µg/mL for
clarithromycin, rifabutin, and ethambutol,
respectively).

The initial description of \textit{M. yon-
gonense} noted that it has a distinct
\textit{rpoB} sequence \cite{1}, identical to that
of a distantly related scotochromo-
genic species, \textit{M. parascrofulaceum}.
In a more recent article \cite{6}, the same
authors investigated 2 more strains of
\textit{M. yongonense} with similar charac-
teristics and suggested that the recent
acquisition of the \textit{rpoB} gene resulted
from a lateral gene transfer event
from \textit{M. parascrofulaceum}. The \textit{rpoB}
genes of the strains we investigated,
however, were substantially different
from that of \textit{M. scrofulaceum} and
were instead related to that of \textit{M. in-
tracellulare} (99.4% similarity) and,
less closely, to that of other species
belonging to the MAC, including \textit{M. marseillense} (97.4%). Discrepancy in
the \textit{rpoB} sequence means some un-
certainty remains that our strains are
\textit{M. yongonense}, but the 100% identity
in major phylogenetically relevant re-
grions strongly supports this hypoth-
esis and suggests the possibility of a
variant of the species preceding the
acquisition of the \textit{rpoB} gene from \textit{M. parascrofulaceum}. Less evidence ex-
ists for identifying the strains as
\textit{M. marseillense} because of the clear di-
vergence in the genes investigated,
other than 16S rRNA.

The complete epidemiology of \textit{M. yongonense} is unknown, in part be-
cause few strains have been identified.
However, as in the cases we describe,
use of suboptimal identification meth-
ods may mean that some isolates have
been misidentified as other mycobac-
teria species.
GenBank accession numbers for the M. yongonense strains identified in this study (FI-13004 and FI-13005) are KF224989–KF224999.

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Subcutaneous Infection with Dirofilaria spp. Nematode in Human, France

To the Editor: The article by Foissac et al. titled Subcutaneous infection with Dirofilaria immitis nematode in human, France (1) presents an interesting and challenging diagnostic dilemma. The paper described, but did not illustrate, the worm as having a strongly ridged external surface of the cuticle—a feature known not to exist on Dirofilaria immitis, the dog heartworm. However, molecular sequencing of the specimen demonstrated much closer similarity to D. immitis than to D. repens, the most common cause of zoonotic subcutaneous dirofilariasis infection in Europe.

Well-described morphologic features of parasites, including in tissue sections, have long been the standard for diagnosis. More recently, molecular diagnostics have helped in many of these difficult cases. However, in some cases, the morphology and molecular diagnosis are discordant. On the basis of the data in the article, the worm does not seem to represent D. repens. A more likely possibility is some other species for which no sequences are yet available for comparison. In such a worm, the regions sequenced must be similar to D. immitis, and distinct from D. repens, to achieve the observed results.

When one encounters a case such as this, where well-validated morphologic features (Figure) are contradictory to the molecular analysis, one must exercise caution in arriving at a final diagnosis. One disadvantage