We describe a 35-year-old man with X-linked agammaglobulinemia who had refractory chronic pleurisy caused by a *Helicobacter equorum*-like bacterium. Broad-range bacterial PCR targeting the 16S and 23S rRNA genes and *in situ* hybridization targeting the 16S rRNA gene of *H. equorum* confirmed the presence of this pathogen in a human for the first time.
using a digoxigenin-labeled single-strand RNA probe. The probe for positions 999 to 1118 in the 16S rRNA gene of *H. equorum* (GenBank accession no. AM998804) was designed as follows:

\[
5'\text{-}H11032\text{-}UCCUCACCUUCCUCCUACGAGGAGCAGCUCCUUAAGGACGAGGGUUGCGCUCGUUGCGGGACUUAACCCAACUUCACGACACGAGC-3' \text{/H11032}
\]

Positive signals in the pleural sample were confirmed with a nitroblue tetrazolium/5-bromo-4-chloro-3-indolylphosphate (NBT/BCIP) system (Roche Diagnostics, Tokyo, Japan) (Fig. 3B) but not in the control, which included the sense probe (Fig. 3C). These results indicated that the refractory chronic pleurisy in our patient was caused by an *H. equorum*-like bacterium, which in turn caused the development of secondary OP.

We began administration of PAPM/BP at a high dose of 8 g/day and of clarithromycin orally for 2 months. Since then, the patient has had no symptoms, and tests have shown negative CRP results and an endotoxin level of less than 10 ng/liter.

Since the discovery of *Helicobacter pylori* in 1984 (7), various *Helicobacter* species have been described in a wide variety of animal hosts, and transmission to humans has been suggested (3, 10, 14). In general, *Helicobacter pylori* is associated with gastritis, peptic ulcer disease, gastric adenocarcinoma, and mucosa-associated lymphoid tissue lymphoma (3). Also, non-*H. pylori* *Helicobacter* species are associated with gastric, intesti-

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**FIG. 1.** Molecular analysis of the BTK gene and pedigree of our patient. (A) A missense mutation, L111R in BTK gene, identified in our patient. (B) The family tree for our patient. An arrow indicates the proband with XLA. The solid squares denote patients with XLA; circles with black dots denote mutation carriers.

**FIG. 2.** Imaging and histological findings of refractory chronic pleurisy and secondary OP. (A and B) Results of a chest radiograph (A) and a chest CT scan (B) upon the latest admission of the patient to the hospital. (C) Histological finding in alveolar spaces, showing intraluminal fibrosis (arrow) (hematoxylin and eosin). (D) Histological finding in the right pleura, showing chronic inflammation (hematoxylin and eosin).
in situ species (3). We therefore performed in general, particularly for non-
the unexpected bacterium, but such culture is also difficult to
species could not be performed for our patient because of
pleures of our patient. Unfortunately, a culture for the
bacterium that has not previously been reported to have been
found in samples from humans was isolated from biopsy sam-
plies (3). Herein, we have also described a case of refractory
chronic pleurisy caused by an H. equorum-like bacterium that
was subjected to molecular analysis.

Our patient had XLA, which is a rare genetic disorder of
B-cell maturation characterized by the absence of mature B
cells, very low serum levels of all immunoglobulin isotypes, and
a lack of specific antibody production (6). He suffered for 2
years from right chronic pleurisy due to an unknown pathogen.
We treated him with PAPM/BP on the basis of the clinical
findings, but we were confused because the efficacy was tran-
sitory. Molecular diagnosis targeting bacterial 16S and 23S
rRNA genes revealed that only DNA of an H. equorum-like bacterium that has not previously been reported to have been
found in samples from humans was isolated from biopsy sam-
plies of our patient. One-tenth the amount used in S1 was used in S2. N, negative control; M, marker. (B) Result of in situ hybridization using
the probe for the 16S rRNA gene of H. equorum. Scale bar, 500 μm. (B') Higher magnification of the
bracketed areas shown in panel B. The signals of H. equorum were detected (arrowheads). Scale bar, 100 μm. (C) Negative control. Scale bar, 500 μm.

FIG. 3. Detection of H. equorum-like bacterium DNA in samples from the right pleura. (A) Broad-range bacterial products from a PCR
targeting the 16S rRNA gene (left) and the 23S rRNA gene (right) determined using biopsy samples from the right pleura. S1 and S2 denote DNA
samples from our patient. One-tenth the amount used in S1 was used in S2. N, negative control; M, marker. (B) Result of in situ hybridization of
the pleural samples performed using the probe for the 16S rRNA gene of H. equorum. Scale bar, 500 μm. (B') Higher magnification of the
bracketed areas shown in panel B. The signals of H. equorum were detected (arrowheads). Scale bar, 100 μm. (C) Negative control. Scale bar, 500 μm.

nal, and hepatobiliary diseases in humans (3, 10, 14). This understanding is attributed to molecular diagnosis based on
the sequencing of bacterial 16S and 23S rRNA genes, an an-
alytical technique that has already proved useful for various bacterial infections during antimicrobial treatment (11), for
rare or unexpected pathogens (11), and particularly for diffi-
cult-to-culture bacteria such as non-H. pylori Helicobacter spe-
cies (3). Herein, we have also described a case of refractory
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found in samples from humans was isolated from biopsy sam-
plies of our patient. Unfortunately, a culture for the Helicobac-
ter species could not be performed for our patient because of
the unexpected bacterium, but such culture is also difficult to
perform in general, particularly for non-H. pylori Helicobacter species (3). We therefore performed in situ hybridization using
the probe for the 16S rRNA gene of H. equorum and thereby
confirmed that the infection had been caused by an H. equo-
rum-like bacterium.

H. equorum, which is a Gram-negative, curved, and motile
bacterium, was recently isolated from horse feces by molecular
diagnosis (8). Additional investigation revealed that the prev-
ance of H. equorum was significantly higher in horses under
veterinary care than in healthy horses, and H. equorum DNA
has never been detected in human samples (9). To the best of
our knowledge, this is the first case of infection with H. equo-
rum-like bacterium in a human with XLA and in the respira-
tory system. So far, Helicobacter infections in patients with
XLA have rarely been reported (2, 4, 12, 13), and none of
those reported have been due to the presence of Helicobacter
species in the respiratory system. Freeman and Holland illus-
trated the importance of humoral immunity in Helicobacter
infections involving mucosal surfaces, because patients with
XLA have been prone to chronic bacteremia, skin infections,
and bone infections by the Helicobacter species (1). Our patient
with XLA showed no evidence of bacteremia or other infec-
tions due to the presence of an H. equorum-like bacterium. In
addition, the studied patient had not had any contact with
horse feces, which is a possible vector of H. equorum, for the
previous 2 years, though he had a history of right pleurisy.
Finally, the source of the infection in our patient could not be
identified, but we think it would be accurate to say that this
infection, which exhibited abnormal humoral immunity, may
have been associated with XLA.

Our patient with XLA has been treated with PAPM/BP, but
we are unsure as to which antimicrobial treatment to use in a
case like this. Because of the difficulty of performing culture, in
vitro susceptibility testing has scarcely been evaluated or stan-
dardized for H. equorum. Moyaert et al. reported resistance to
cephalotin and nalidixic acid and sensitivity to metronidazole
for H. equorum (8). We also noted evidence of multiple drug
resistance of this organism clinically, as our patient improved
only after treatment with PAPM/BP; administration of many
other antimicrobial treatments resulted in no improvement.
Further investigation is needed, because antimicrobial treat-
ment for H. equorum may be difficult.

In conclusion, we have described a case of chronic pleurisy
associated with the presence of an H. equorum-like bacterium.
All of the clinical findings for our patient—transient PAPM/BP effectiveness, a high serum endotoxin level, and imaging-histological findings of chronic inflammation—were consistent with infections by this organism. This case illustrates both the usefulness of molecular diagnosis of infections with unknown organisms and the pathogenicity of the *H. equorum*-like bacterium in immunocompromised humans. In the future, the issues of whether *H. equorum* is associated with diseases in immunocompetent humans or not and of how patients infected with *H. equorum* are to be treated need to be investigated.

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