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First Human Systemic Infection Caused by *Spiroplasma*

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***Spiroplasma* species are organisms that normally colonize plants and insects. We describe the first case of human systemic infection caused by *Spiroplasma* bacteria in a patient with hypogammaglobulinemia undergoing treatment with biological disease-modifying antirheumatic agents. *Spiroplasma turonicum* was identified through molecular methods in several blood cultures. The infection was successfully treated with doxycycline plus levofloxacin.**

CASE REPORT

A 73-year-old Caucasian woman presented to the emergency room complaining of a 2-month history of intermittent fevers of up to 38°C, proximal myalgias, a frontal headache, apathy, fatigue, and progressive swelling of the limbs. Physical examination revealed bilateral conjunctival injection. Heart murmurs were not detected, and the remainder of the examination was also normal.

The patient had a history of rheumatoid arthritis and for the last 10 years had been receiving continuous biological disease-modifying antirheumatic agents for frequent flare-ups. The biologic agents used had sequentially included etanercept, rituximab, and tocilizumab. The latter had been given for the last 3 years, until it was replaced with certolizumab 3 months before admission because of inadequate pain control. Other relevant clinical data included a selective IgM deficiency diagnosed 1 year before, with undetectable serum IgM levels, and long-term cholestatic liver disease of unknown etiology. The patient lived in an urban setting, and she denied contact with plants or animals or insect bites. There was no other epidemiological or personal background of interest.

On admission, her blood tests revealed microcytic anemia suggestive of chronic disease (hemoglobin concentration, 10 g/dl [reference range, 12 to 15.5 g/dl]). Acute-phase reactants were elevated, with a C-reactive protein level of 141 mg/dl (reference range, <5 mg/dl), an erythrocyte sedimentation rate of 103 mm/h (reference range, 0 to 10 mm/h), and a fibrinogen level of 768 mg/dl (reference range, 150 to 500 mg/dl). The hepatic cholestasis enzymes were increased, similar to the patient's usual levels, with a normal bilirubin concentration. Serum IgM was undetectable (<5 mg/dl [reference range, 60 to 250 mg/dl]), and levels of IgG were low (304 mg/dl [reference range, 680 to 1,530 mg/dl]). Her serum was negative for markers of autoimmunity, the usual serum tumor markers, rheumatoid factor, and anti-citrullinated peptide antibodies. Urinalysis and serum and urine electrophoresis results were unremarkable. Thoracic computed tomography (CT) revealed mild bilateral posterobasal pleural effusion with bilateral infiltrates in "frosted glass" suggestive of heart failure. Cranial, abdominal, and pelvic CT did not show abnormal findings.

Two blood culture sets were drawn. Each blood culture set included an aerobic and an anaerobic bottle (Bactec 9240; Becton-Dickinson Diagnostic Instrument Systems). Empirical antibiotic therapy with intravenous cefuroxime was started on admission. After 48 h of incubation, the two blood culture sets became positive without microscopic evidence of growth by Gram stain. Two additional sets of blood cultures were drawn that were also positive without microscopic evidence of growth. Forty-eight hours later, subcultures on blood agar plates showed several small zones of beta-hemolysis with no macroscopic colony growth and with absence of organisms upon Gram, Giemsa, and Ziehl-Neelsen staining. Additional direct staining of blood cultures including Giemsa, Kinyoun, and Ziehl-Neelsen stains was also negative. Subcultures of blood cultures in a wide variety of culture media, including media for *Legionella*, *Mycoplasma* (A7 solid medium), *Campylobacter*, anaerobic bacteria, and fungi, were all negative. Blood was also subcultured in thioglycolate broth and again processed for culture after 2 days in all of the previously mentioned media. A blinded sample from the zone of hemolysis of blood agar plates was additionally subcultured in those media. All of the cultures were negative, except the subculture on the solid A7 agar of the zone of hemolysis of blood agar. HACEK microorganisms (*Haemophilus* species, *Aggregatibacter actinomycetemcomitans*, *Cardiobacterium hominis*, *Eikenella corrodens*, and *Kingella kingae*) and probiotic-related organisms (*Bacillus*, *Lactobacillus*, *Saccharomyces*, and *Bifidobacterium*) were not recovered after culture in different incubation atmospheres and in different culture media, including blood agar, chocolate agar, MacConkey agar, Wilkins-Chalgren agar, and Sabouraud agar. *Borrelia*, *Bartonella*, *Chlamydia*, *Legionella*, *Tropheryma whipplei*, *Coxiella*, and mycobacteria were ruled out by molecular and/or serological methods. PCR with hybridization was used at the Spanish National Center for Microbiology (Instituto de Salud Carlos III, Madrid, Spain) for *Coxiella burnetii*, *T. whipplei*, and *Bartonella*. The FluoroType MTB PCR assay and Lowenstein and Middlebrook 7H9 broth cultures were used for *Mycobacterium tuberculosis*. Positive blood culture bottles and blood agar plates were sent to the reference laboratory of taxonomy of the Spanish National Center for Mi-

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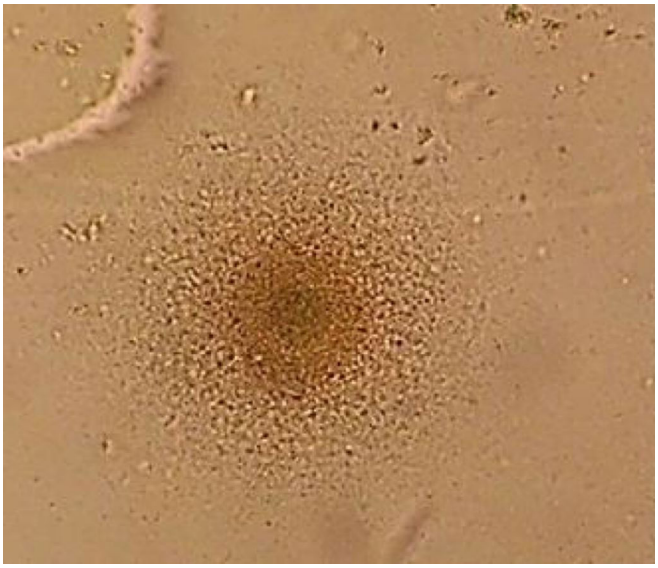

FIG 1 Growth of *Spiroplasma* bacteria on *Mycoplasma* A7 agar medium. Magnification, ×40.

crobiology (Instituto de Salud Carlos III, Madrid, Spain) for identification.

Subculture on solid medium for the identification of *Mycoplasma*/*Ureaplasma* (A7 agar medium) by the zone of hemolysis visualized on blood agar yielded some colonies with the appearance of *Mycoplasma* (Fig. 1). A7 agar is a highly nutritive growth medium containing peptones, horse serum, and growth factors (cysteine, Polyvitex, arginine, and urea) that favors the development of *Mycoplasma* and *Ureaplasma* colonies. The medium contains an antibiotic mixture for the inhibition of Gram-positive and Gram-negative bacteria (bioMérieux package insert).

Sequencing of the colonies isolated on A7 agar medium was performed by PCR. A 1,482-bp region between positions 8 and 1,492 of the bacterial 16S rRNA was amplified from 1 μl of DNA isolated with Chelex-100 (Bio-Rad, Hercules, CA). The amplification reaction took place in a GeneAmp PCR system 9700 thermal cycler (Applied Biosystems, Foster City, CA) with a high-fidelity enzyme, Extensor Long Range PCR Enzyme (Thermo Scientific, Leicestershire, United Kingdom), and universal 16S rRNA primers 27F (AGAGTTTGATCMTGGCTCAG) and 1492R (GYTACC TTGTTACGACTT), and the amplification protocol was 94°C for 2 min, followed by 30 cycles of 94°C for 10 s, 50°C for 30 s, and 68°C for 90 s and a final step of 68°C for 7 min. After amplification, the PCR products were visualized in a 1% agarose gel stained with ethidium bromide. A partial 16S rRNA sequence of 1,302 bp was obtained. All PCR products were sequenced (Secugen, Madrid, Spain) in the forward and reverse directions and assembled previous to taxonomic assignment on the basis of the available 16S rRNA databases, GenBank, and the Seqmatch tool. Sequence comparisons using the Ribosomal Database Project II website to determine the best match to known sequences by considering only sequence similarity (S_ab) scores of >0.99 identified our isolate as a member of the genus *Spiroplasma*, and the highest match (S_ab score = 0.995) corresponded to *Spiroplasma turonicum* ATCC 34211. By the above-mentioned procedures, *Spiroplasma* was finally identified in five blood culture sets. The reference laboratory of taxonomy also identified *Spiroplasma* species in the samples submitted.

Transthoracic and transesophageal echocardiography showed no evidence of endocardial vegetation. The patient remained febrile while on antibiotic therapy with cefuroxime for 2 weeks, and her fever persisted after she was switched to piperacillin-tazobactam. Her fever did not subside either after biological disease-modifying antirheumatic therapy with tocilizumab was restarted because of uncontrolled articular disease. With the preliminary report of the isolation of a *Mycoplasma*-like organism, a new regimen with doxycycline plus levofloxacin was started. The patient became afebrile in the following 72 h, and her clinical condition improved. Her acute-phase reactant levels returned to normal values, and her anemia resolved within the following weeks. In addition to antibiotics, she received intravenous immunoglobulins for her IgM and IgG deficiency. Combined antibiotic therapy was maintained for 2 months. The patient remained afebrile throughout the treatment period. Three sets of two blood cultures taken during and after treatment were negative. To date, 1 year after admission, the patient remains asymptomatic.

Spiroplasma is a helical member of the class *Mollicutes* and the family *Spiroplasmataceae* that colonizes or infects plants and insects (1). The members of the class *Mollicutes* are the smallest bacteria yet described. The majority of them are facultatively anaerobic, pleomorphic prokaryotes that lack a cell wall and are not sensitive to beta-lactams (2). *Spiroplasma* species are fastidious organisms that require enriched culture media and are difficult to grow in culture, with variable growth times. The genus *Spiroplasma* was discovered relatively recently (3). The first species was described in 1972 as the etiological agent of a plant disease ("stubborn disease") (3, 4), although in 1961, Poulson and Sakaguchi (5) had already observed by microscope the first spiroplasma (thought at the time to be a spirochete) as the causative agent of the "sex-ratio" disease of *Drosophila* flies. *Spiroplasma* was elevated to the generic name of this group of microbes and added to the Approved Lists of Bacterial Names in 1983 (6). The major reservoir of spiroplasmas are insects (7, 8), and the surfaces of flowers and other plant parts are the major sites of their acquisition and transmission (9, 10). Spiroplasmas show many of the properties of mycoplasmas, including the formation of typical colonies with a "fried-egg" appearance on solid media but with cells with helical mobility in liquid culture media (2, 3).

To our knowledge, this is the first documented case of systemic infection caused by *Spiroplasma* bacteria in a human, which was manifested as continuous bacteremia with five positive sets of blood cultures. To date, the only case of isolation of a *Spiroplasma* species from a human had been reported in the conjunctiva of a premature newborn with a unilateral cataract associated with anterior uveitis (11). *Spiroplasma* species have also been linked by molecular and serological studies to the pathogenesis of transmissible spongiform encephalopathies (12).

Of interest, our patient was undergoing long-term therapy with biological disease-modifying antirheumatic agents. Anti-tumor necrosis factor alpha antibody and other agents targeting key components of the immune system leave the patient more susceptible to infection by inducing a certain degree of immunosuppression. Besides mycobacterial infections, several serious emerging

infections are increasingly being described in patients undergoing biological therapies, including infections caused by other intracellular pathogens such as *Legionella*, *Listeria*, and *Leishmania* and by endemic fungi and reactivation of viral infections, including hepatitis, herpesviruses, and more recently the polyomavirus JC (13). *Spiroplasma* might represent a new opportunistic emerging pathogen associated with biologics. Likewise, hypogammaglobulinemia has been linked to increased susceptibility to *Mycoplasma* and *Ureaplasma* species infections (14) and might also have contributed to the acquisition of the infection of our patient.

Our patient received combination antibiotic therapy with doxycycline and levofloxacin. Tetracyclines are considered the drugs of choice for *Spiroplasma* infections (15). However, the therapeutic activity of tetracyclines in *Mycoplasma* infections may become unreliable because of resistance phenomena (16) induced by ribosomal modification, and their bacteriostatic activity is also a drawback for a systemic infection with a chronic course. We chose the combination of doxycycline with levofloxacin because our patient was immunocompromised. Fluoroquinolones are bactericidal antibiotics that are frequently active against *Mycoplasma hominis* (17), a pathogen phylogenetically related to *Spiroplasma*; no antagonism has been described for the combination; and an additive effect *in vitro* against certain infections was even found (18). The clinical and microbiological resolution in our patient suggests that this regimen might be a suitable therapeutic alternative for serious systemic infections, although further clinical experience is needed to confirm our observation.

Spiroplasma represents a new genus in human infectious pathology. Whether it could be a new emerging pathogen associated with infections in immunocompromised humans remains to be seen. Clinicians and microbiologists should be aware of this pathogen, which requires specific culture media and molecular methods for identification and targeted antibiotic therapy for eradication.

Nucleotide sequence accession number. Our strain's DNA sequence has been deposited in GenBank under accession number KJ596453.

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