BRIEF REPORT

# Recurrent Bacteremia and Multifocal Lower Limb Cellulitis Due to *Helicobacter*-Like Organisms in a Patient with X-Linked Hypogammaglobulinemia

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**We describe a 27-year-old man with X-linked (Bruton's) hypogammaglobulinemia who presented during a 10-month period with recurrent fevers and multifocal lower-limb cellulitis associated with bacteremia due to *Helicobacter*-like organisms *("Flexispira rappini"* and *Helicobacter canis).* Susceptible individuals may acquire infection of this type as a result of exposure to young dogs.**

During the past 2 decades, ⩾18 species belonging to the genus *Helicobacter* have been isolated from human and veterinary sources [1]. In humans, specific immunodeficiency states appear to be associated with invasive infection with *Helicobacter* species. Patients with AIDS, for example, are prone to invasive infection with *Helicobacter cinaedi* [2]. Recently, there have been 2 case reports of invasive infection with an organism resembling a *Helicobacter* species with the vernacular name "*Flexispira rappini*" in patients with X-linked hypogammaglobulinemia [3–4]. We report an additional case that, on this occasion, was associated with coinfection with *Helicobacter canis.*

***Case report.*** The patient was a 27-year-old man who had X-linked hypogammaglobulinemia diagnosed during childhood. He received monthly iv Ig and, although he had mild bronchiectasis, he remained relatively free of symptoms until 1998. He owned a 9-month-old German shepherd puppy at that time.

During a 10-month period (December 1998–September 1999), the patient experienced recurrent episodes of fever. These episodes were associated with distal lower-limb pains, usually with 1 or 2 patches of cellulitis appearing below the knees. The patches were 5–10 cm in diameter. On one occasion, in August 1999, the fevers were associated with left flank pain and dysuria. The patient was admitted to the hospital on 6 occasions during this period.

From December 1998 through July 1999, the patient was treated empirically with repeated courses of iv and oral $\beta$-lactam antibiotics for varying periods. Antibiotics used at this stage included penicillins (benzylpenicillin, amoxycillin, flucloxacillin, and dicloxacillin) and a 5-week course of iv ceftriaxone.

In August 1999, because a gram-negative organism had been identified in blood cultures, the patient was treated initially with iv gentamicin and ampicillin for 1 week followed by oral ciprofloxacin for 4 weeks. Unfortunately, patches of lower-limb cellulitis associated with pain, fever, and gram-negative bacteremia recurred while the patient was still taking ciprofloxacin.

A cure was achieved only after the patient commenced a 5-month course of oral doxycycline (100 mg b.i.d) and metronidazole (400 mg b.i.d.) in September 1999. Symptoms resolved within a few days after commencement of this treatment, and they have not returned after >18 months.

***Materials and methods.*** Bacterial isolates were recovered from blood samples by use of the BacT/Alert Microbial Detection System (Organon Technika). Positive bottles were subcultured onto trypic soy agar with 5% sheep blood and chocolate agar (bioMérieux Australia). Plates were incubated aerobically in an atmosphere of 5% $CO_2$ and anaerobically in an atmosphere of 10% $CO_2$ and 10% hydrogen in nitrogen at 35°C for a period of at least 72–96 h. Single colonies were further subcultured for gene sequencing. Biochemical tests were performed using standard methods.

DNA from the isolates was extracted using the QIAamp DNA Mini Kit (Qiagen) according to the manufacturer's instructions. Genotypic identification was achieved by using PCR to amplify a trimmed portion of the 16S rRNA gene of each isolate. The PCR mixture contained 10 ng of genomic DNA, 10 pmol each of universal 16S rRNA primers 515FPL (5′-TGC CAG CAG CCG CGG TAA–3′) and 13B (5′-AGG CCC GGG AAC GTA TTC AC–3′) [5], 200 $\mu$M dNTPs, 1.5 U platinum *Taq* polymerase (Life Technologies, Gibco BRL), and 1.5 mM $MgCl_2$ in a total volume of 50 $\mu$L. The PCR reaction had an initial denaturation of 94°C for 3 min with a subsequent 35-cycle amplification consisting of denaturation at 94°C for 30 s, annealing at 54°C for 30 s, and extension at 72°C for 1 min. PCR products were visualized on a 1% agarose gel that contained 0.5 $\mu$g/mL

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ethidium bromide. The products were purified using Centricon 100 filters (Millipore) and directly sequenced twice in both directions using the ABI 377 sequencer after dye terminator cycle sequencing (ABI Prism BigDye terminator sequencing kit; Perkin-Elmer USA) according to manufacturers' instructions. A consensus sequence was derived and analyzed using the National Center for Biotechnology Information (NCBI) Basic Local Alignment Search Tool (BLAST).

***Results.*** Of 17 sets of blood cultures (34 bottles), unusual gram-negative organisms grew in 4 aerobic bottles (table 1). The first of these was in May 1999, but, although a spiral organism was seen on a Gram stain of the blood culture fluid, the organism failed to grow in subculture.

In August and September 1999, gram-negative organisms were successfully subcultured from 3 blood culture bottles. The isolates were oxidase positive and catalase negative. Curiously, however, 2 different *Helicobacter*-like organisms were identified, which most closely resembled *H. canis* and "*F. rappini*" genotypically. The initial subculture of the blood obtained in August apparently yielded a mixed growth of the 2 organisms. Spiral organisms with morphology consistent with *H. canis* were identified on a Gram stain, but a strong urease reaction that was more typical of "*F. rappini*" was demonstrated from the primary inoculum of the subculture. A single colony from this culture was further subcultured and identified. Phenotypically, this organism demonstrated spiral morphology, but, unlike the primary inoculum, it was urease negative. The 16S rRNA sequence analysis showed that this organism was highly similar (99.6%) to *H. canis*. In September 1999 (while the patient was receiving ciprofloxacin), a urease-positive organism was isolated from blood cultures in pure growth. This time the organism had a fusiform appearance that was typical of "*F. rappini.*" The 16S rRNA sequence analysis revealed that this isolate was highly similar (99.7%) to "*F. rappini.*"

***Discussion.*** In recent years, *Helicobacter* species have been recognized with increasing frequency as causes of human infection. With the exception of *H. pylori,* however, these organisms are considered to be zoonotic pathogens [6].

Recognition of invasive *Helicobacter* infection is hampered by 2 main problems: (1) they are fastidious organisms that may fail to grow in primary culture or subculture [7], and (2) like other *Campylobacter*-like organisms, they are relatively inert biochemically, which makes identification extremely difficult without resorting to gene sequencing techniques.

It is now clear that patients with X-linked (Bruton's) hypogammaglobulinemia are susceptible to infection with organisms of this type, and "*F. rappini*" in particular. There have been 2 previous case reports of invasive "*F. rappini*" infections in such patients. In 1999, Weir et al. [3] described a 36-year-old man with X-linked hypogammaglobulinemia who presented with persistent "*F. rappini*" bacteremia associated with lower-limb edema and cellulitis. More recently, researchers from Germany [4] described a 29-year-old man who had X-linked hypogammaglobulinemia with recurrent abdominal abscesses from whom a *Helicobacter* species closely related to "*F. rappini*" was isolated.

"*F. rappini*" has also been isolated from patients *without* X-linked hypogammaglobulinemia. Case reports in the literature include a child with pneumonia [7], an adult patient who was undergoing hemodialysis [6], and 2 patients with mild chronic diarrhea [8].

As the name implies, *H. canis* has primarily been isolated from dogs (with and without diarrhea) [9]. There has been 1 previously published report of *H. canis* infection in a human, which involved a boy with gastroenteritis [10] and no previous associations with X-linked hypogammaglobulinemia.

We hypothesize that the patient in the present report acquired the *H. canis* infection from his recently purchased puppy.

**Table 1. Blood culture findings.**

| Date samples were obtained | Positive blood cultures; Gram stain | Primary subculture findings | Secondary subculture of a single colony findings |
|---|---|---|---|
| December 1998 | 0/2 bottles | — | — |
| January 1999 | 0/2 bottles | — | — |
| February 1999 | 0/2 bottles | — | — |
| May 1999 | 1/4 bottles; spiral organism | No growth | — |
| August 1999 | 2/10 bottles; spiral organism | Spiral organism urease positive (mixed growth?) | Spiral organism urease negative (genotypically resembling *Helicobacter canis*) |
| September 1999[a] | 1/4 bottles; fusiform bacillus | Fusiform bacillus urease positive | Fusiform bacillus urease positive (genotypically resembling *"Flexispira rappini"*) |
| November 1999[b] | 0/2 bottles | — | — |
| February 2000 | 0/2 bottles | — | — |

[a] Sample was obtained while the patient was receiving oral ciprofloxacin.
[b] Sample was obtained while the patient was receiving oral doxycycline and metronidazole.

In light of the demonstration of coinfection with *"F. rappini,"* we suggest that the latter organism also originated from the young dog. There is evidence in the literature to support this contention. In 3 of the 6 reports of human infection with *"F. rappini"* that have been published elsewhere, exposure to young dogs was documented [6–8]. In 1 of these cases [8], the same organism was also recovered from a 5-month-old family dog but not from an older dog. In other reports, the organism has been isolated from aborted sheep fetuses [11] and mouse intestinal mucosa [12]. Whether there is another reservoir of infection to account for cases without documented dog exposure is not clear.

Although men with X-linked (Bruton's) hypogammaglobulinemia usually lead productive adult lives, they are prone to certain specific infections, even when they are receiving regular Ig replacement. Severe invasive infections with bacteria from the genus *Campylobacter* are well known to be associated with this condition. Commercially prepared Ig has been shown to fail to correct the inability of such patients' serum to opsonize these organisms [13]. In light of the close phylogenetic relationship between *Campylobacter* and *Helicobacter* species, it seems likely that there is a similar mechanism of immunodeficiency at play in infections of both types. Therefore, this type of infection may not be seen in cases in which hypogammaglobulinemia is due to other causes.

The clinical picture of recurrent fever and multifocal cellulitis seen in this case closely resembles that described by Weir et al. [3] in a similar patient with X-linked hypogammaglobulinemia and bacteremia due to a *"Flexispira"*-like organism. Detection of bacteremia may require repeated collection of blood for culture, possibly over months. Even if the organism grows in the liquid blood culture medium, it may fail to grow in subculture. The clinician, therefore, may perform a critical role in recognizing the syndrome, thus facilitating the identification of the pathogen in the laboratory.

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