"Campylobacter cinaedi" Bacteremia: Case Report and Laboratory Findings

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"Campylobacter cinaedi" was isolated from the blood of a 29-year-old homosexual man with previously diagnosed acquired immune deficiency syndrome. Subculturing of the organism was achieved with the use of 7% lysed horse blood and 10% sheep blood agars at 37°C in a microaerophilic atmosphere. Problems associated with the culturing of this organism are reviewed.

The association between Campylobacter-like organisms and enteric disease in homosexual men has been previously described (5, 6). These campylobacters are genotypically distinct from Campylobacter fetus and Campylobacter jejuni, and DNA homology studies have divided them into four different groups (1, 8). Two of these groups have been tentatively given species recognition and named "Campylobacter cinaedi" and "Campylobacter fennelliae" (8).

Bacteremia from Campylobacter-like organisms subsequently identified as "C. cinaedi" has previously been noted in two patients elsewhere (4). We report here the documentation of a third case and discuss the clinical and laboratory features pertinent to its recognition.

A 29-year-old male homosexual was hospitalized in March 1986 with a 48-h history of fever and chills. Two years prior to admission, the patient was first noted to have generalized lymphadenopathy, and 12 months later, a diagnosis of acquired immune deficiency syndrome was made on the basis of positive human immunodeficiency virus serology and the presence of an undifferentiated lymphoma. The patient had subsequently received intermittent chemotherapy. He had also received co-trimoxazole as prophylaxis against infection.

On admission, the patient complained of fever, chills, and a mild cough productive of scant yellow sputum. He described abdominal distention but did not have diarrhea, rectal irritation, or discharge. Other symptoms included a mild dysuria and tenderness of his left testicle. He participated in both active and passive roles including oral-anal sex in a monogamous homosexual relationship without adhering to safe sexual practices.

A physical examination revealed a temperature of 39.5°C, a pulse rate of 112 beats per min, a respiratory rate of 12 breaths per min, and a blood pressure of 120/80 mm Hg (ca. 200 Pa). There was no significant lymphadenopathy, but he did have moderate oral candidiasis. The left testicle was tender. Initial laboratory data revealed a leukocyte count of 6.2 × 10⁹/liter, with 90% granulocytes, 13 g of hemoglobin per liter, and a platelet count of 80 × 10⁹/liter.

After samples were taken from blood, sputum, stool, urine, and urethra for culturing, tobramycin and cefazolin were commenced in view of his septic presentation. Tetracycline was added because of symptomatic epididymitis.

Within 24 h of hospitalization, the chills and fever had resolved, but four loose stools containing blood were passed. This diarrhea resolved within another 24 h, although two loose, nonbloody stools were passed in the next 48 h. Sputum and urine cultures were negative. Stool and rectal swabs were negative for Salmonella spp., Shigella spp., Campylobacter spp. (Butzler selective medium), Yersinia enterocolitica, Aeromonas spp., Escherichia coli 157, ova and parasites (including Cryptosporidium spp.), Clostridium difficile toxin, and Neisseria gonorrhoeae. After 6 days, two aerobic blood cultures drawn on admission gave low positive radiometric readings (BACTEC), and Gram staining failed to reveal organisms. However, acidine orange staining demonstrated spiral gull-wing forms compatible with Campylobacter species. Two anaerobic bottles (BACTEC) were negative. Oral erythromycin for 1 further week was substituted for tobramycin and cefazolin, and the patient was discharged well on hospital day 7. He was well at follow-up 6 months later.

Initial attempts at subculturing the presumed Campylobacter-like organism from the original blood culture bottles were unsuccessful. The bacterium could not be supported by 5% sheep blood agar with Columbia base (Oxoid Ltd., London, England), chocolate agar, buffered charcoal-yeast extract agar (GIBCO Laboratories, Grand Island, N.Y.), 10% sheep blood agar with brucella base (BBL Microbiology Systems, Cockeysville, Md.), Schaedler medium (Oxoid), or Butzler medium (Oxoid) in aerobic, anaerobic, or Campylobacter microaerophilic conditions at either 37 or 42°C. Campylobacter microaerophilic conditions were achieved by evacuate an anaerobic jar twice to approximately 15 to 20 in. Hg (ca. 3.4 kPa) and refilling it with a gas mixture of 10% CO₂–90% N₂. Propagation was achieved by subculturing from the initial aerobic bottles into more aerobic (BACTEC) bottles supplemented with 5 ml of fresh human blood. Culturing on solid media was subsequently achieved by use of 7% lysed horse blood agar with Mueller-Hinton base and 0.1% GC supplement (Alpkem Western Ltd., Calgary, Alberta, Canada). The observation on one occasion of swarming and enhanced growth around a contaminating colony of Bacillus sp. prompted the addition of 0.025% cysteine to 10% sheep blood agar with brucella base, which successfully supported growth. After further subculturing, the organism was capable of growing on the same medium.
without cysteine. Enhanced growth around the Bacillus sp. could not be repeated.

An initial stool collected while the patient was on co-trimoxazole was preserved in Cary-Blair transport medium, kept at 4°C for 1 week, and then, along with a stool collected on follow-up, examined for Campylobacter spp. A membrane filter technique (7) with 10% sheep blood agar in brucella base and a microaerophilic 37°C incubation were used. These cultures, kept for 7 days, did not reveal Campylobacter-like organisms.

The organisms were gram negative and gull-winged and demonstrated corkscrew motility in wet mounts. Electron microscopy revealed single polar flagella. The organism was oxidase and catalase positive and grew in a microaerophilic environment at 37°C but not at 25 or 42°C. No growth occurred in either aerobic or anaerobic conditions at 37°C. The organism neither hydrolyzed hippurate nor produced H2S, as assessed in triple sugar iron slants. It produced H2S, as assessed with lead acetate paper, and reduced nitrate. In a disk diffusion technique, susceptibility to nalidixic acid, cephalothin, erythromycin, and tetracycline was demonstrated. These findings suggested the identification of "C. cinaedi." Analysis of protein profiles by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and Western blotting with "C. cinaedi" rabbit antiserum supported this identification (8). M. Flores, C. L. Fennell, and W. E. Stamm, Program Abstr. 26th Intersci. Conf. Antimicrob. Agents. Chemother., abstr. no. 106, 1986).

In the previous two reported cases of "C. cinaedi" bacteremia, difficulty was encountered in subculturing and identifying this organism. In those cases, high radiometric indices in blood culture bottles (BACTEC) were obtained after 48 h of incubation, but we found only low indices after 6 to 7 days. Our positive cultures were initially only detected by acridine orange staining. Additionally, prolonged incubation upon subculturing on solid media may be required, and certain Campylobacter selective media, e.g., Butzler, may not support growth because of the presence of inhibitory antibiotics, e.g., cephapilo, usually used in C. jejuni selection.

Our patient developed bacteremia while on co-trimoxazole prophylaxis, suggesting resistance to this agent in vivo, a finding supported by the ability to isolate "C. cinaedi" on media containing trimethoprim (1, 4). Results of agar dilution method susceptibility testing on a large number of similar strains agree with these data (2). Disk diffusion studies on 7% horse blood agar in Mueller-Hinton base with GC supplement revealed only faint zones of inhibition with both trimethoprim and co-trimoxazole.

Despite the finding of "C. cinaedi" in stools of homosexual men with proctocolitis, both previous reports documented bacteremia without gastrointestinal symptoms. Our patient developed bloody diarrhea within 24 h of admission, and this resolved while the patient was receiving multiple antibiotics. Although attempts to culture Campylobacter spp. from stools, were unsuccessful, this may have been related to the initial lack of consideration of this pathogen and its particular isolation requirements, which differ from those of thermophilic enteric Campylobacter spp. The failure of the membrane filter technique might well have been related to the nature of the specimens that were available for culturing. The utility of membrane filters for the culturing of Campylobacter-like organisms from homosexual men with enteric diseases requires a prospective evaluation.

We are able to propagate the organism under the previously defined microaerophilic conditions. Other studies have used an atmosphere generated by an anaerobic GasPak (BBL Microbiology Systems) without a catalyst (5, 6). The latter conditions can be potentially hazardous to laboratory workers because of the dangers of hydrogen explosion and increased jar pressure. Although C. jejuni and C. coli may not require hydrogen for growth (3), hydrogen requirements have not been defined for "C. cinaedi" and "C. fennelliae." In this suggested discrepancy between growth in our microaerophilic atmosphere and that generated in a GasPak without a catalyst is secondary to the proportion of hydrogen in the incubation jars, the problems with each system could be rectified by adjusting the hydrogen concentration in the gas mixture used to refill evacuated jars. Further study will be required to define the optimal incubation atmosphere for these organisms.

Wang and Blaser have described problems inherent in the blood culturing of C. jejuni and C. fetus (9). They have demonstrated differences in the ability of these species to grow in blood culture bottles with different atmospheres. Their findings await confirmation in clinical studies before their significance can be fully determined.

Although definitive recommendations for routine culturing of "C. cinaedi" and "C. fennelliae" from patients with diarrhea cannot be made at this time, consideration of these organisms should be maintained in the appropriate clinical context, such as homosexual men with proctocolitis.

LITERATURE CITED


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