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The Brief Case: Postinfectious Glomerulonephritis as an Unexpected Sequela of Drinking Raw Milk

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CASE

A 13-year-old female with a history of Henoch-Schönlein purpura (HSP; IgA vasculitis) at 15 months of age and recurrent urinary tract infections during age 8 to 9 years presented to her primary care physician (PCP) with complaints of 1 day of hematuria, left side flank pain, and a fever of 38.9°C. She was subsequently referred to the emergency department for further care. Her white blood cell (WBC) count was within normal limits (12,000 WBC/μl; reference range, 4,500 to 13,000 WBC/μl), and she had an elevated C-reactive protein level of 4.5 mg/dl (reference range, 0 to 1.0 mg/dl) and creatinine level of 1.1 mg/dl (reference range, 0 to 1.0 mg/dl). A clean-catch urine specimen was sent for urinalysis and culture. The urine was noted to be cloudy and brown with >300 mg/dl protein, 15 mg/dl ketones, and nitrites present. There were >250 red blood cells and 20 to 50 WBCs per high-power field. Her urine culture grew >100,000 CFU/ml of mixed Gram-positive flora, defined in our laboratory as ≥3 organisms without a predominant uropathogen. The urine culture results indicated a contaminated specimen. A single blood culture composed of one aerobic and one anaerobic bottle (VersaTREK REDOX 1 and REDOX 2; Thermo Fisher Scientific, Waltham, MA) was also collected in the emergency department. A renal ultrasound was performed and showed bilateral nonspecific increased echogenicity in the kidneys.

The patient was administered a dose of ceftriaxone and admitted for further evaluation with a presumed diagnosis of pyelonephritis. Ceftriaxone was continued during her admission. The patient's creatinine levels increased to 1.6 mg/dl and 1.9 mg/dl during the 2 days after admission, and her diagnosis was updated to glomerulonephritis. Thirty-two hours after collection, the aerobic blood culture bottle signaled positive. No organisms were seen on Gram stain, so an acridine orange (AO) stain was performed, which revealed curved seagull-shaped rods (Fig. 1A). Upon review of the Gram stain, very faint curved Gram-negative rods were seen (Fig. 1B). The positive blood culture broth was subsequently subcultured per standard protocol to blood agar, chocolate agar, and MacConkey agar. Additionally, due to the suspicion of *Campylobacter* sp. or, less likely, *Helicobacter* sp., the blood culture broth was subcultured to two plates each of cefoperazone-vancomycin-amphotericin B (CVA) agar and chocolate agar. One set of plates was incubated under microaerobic conditions at 42°C (GasPak EZ CampyPouch system; Becton, Dickinson and Company, Franklin Lakes, NJ), and the second set of plates was incubated under anaerobic conditions at 35°C. Growth was only observed on the CVA and chocolate agar plates incubated under microaerobic conditions at 42°C. Colonies were wet and spready, with a slight brown hue (Fig. 2). Colonies were subjected to matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS; Bruker Daltonics, Inc., Billerica, MA), and the isolate was identified as *Campylobacter jejuni*, with scores of 2.19 and 2.21 using the Biotyper-CA claim 2 *in vitro* diagnostic (IVD) library. The identification was reported,

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For answers to the self-assessment questions and take-home points, see https://doi.org/10.1128/JCM.01790-18 in this issue.

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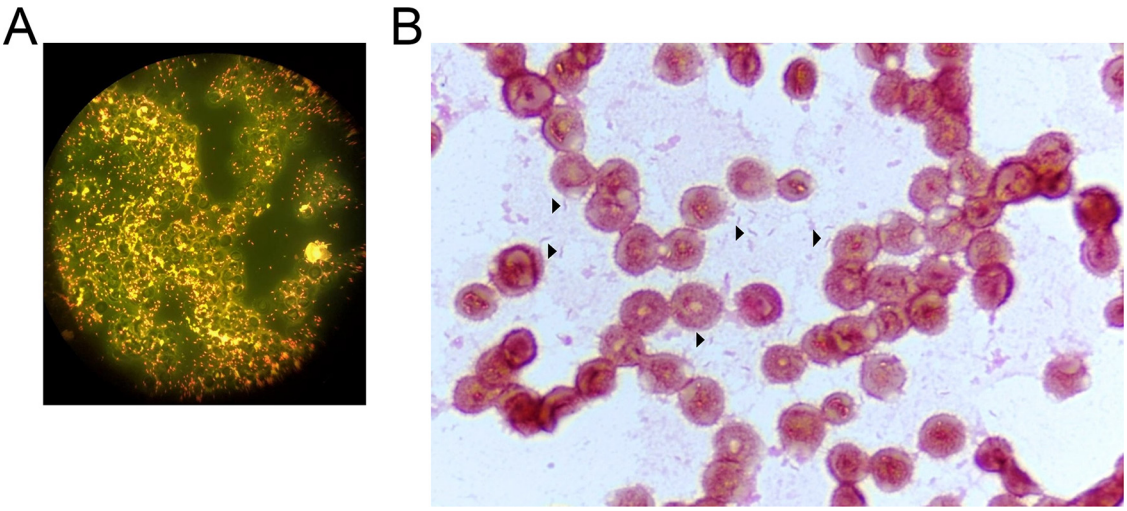

FIG 1 (A) Acridine orange stain of the positive blood culture broth. *Campylobacter* organisms appear bright orange, while human cells and cell debris appear green. (B) Gram stain of the positive blood culture broth. Arrowheads indicate some of the faint curved Gram-negative rods. Total magnification, ×1,000.

which resulted in an infectious diseases consult and the addition of azithromycin to the patient's regimen. The isolate was sent to a reference lab (ARUP Laboratories) for susceptibility testing by broth microdilution; it was susceptible to ciprofloxacin, and the MIC for azithromycin was ≤0.06 μg/ml. Of note, there are no interpretive criteria for azithromycin and *Campylobacter* spp. Azithromycin results can be inferred from testing erythromycin; however, an erythromycin result was not reported by the reference

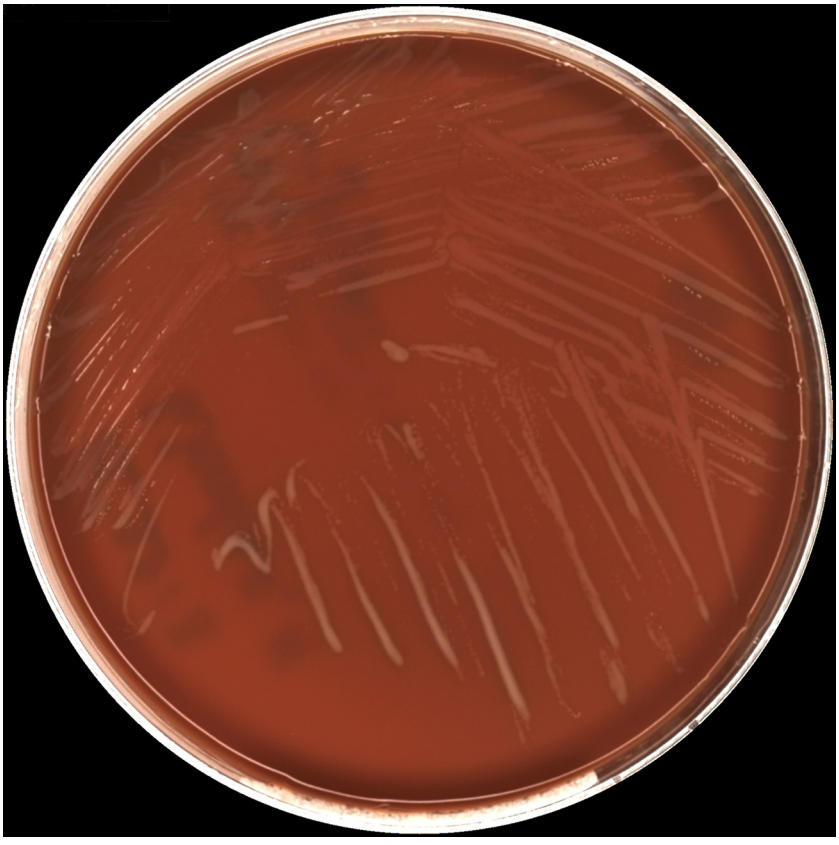

FIG 2 Growth of *C. jejuni* on chocolate agar after incubation at 42°C under microaerobic conditions.

laboratory. No other antimicrobial susceptibilities were reported. The patient was discharged 6 days after admission and received a total of 14 days of azithromycin.

The patient and her mother denied any preceding diarrheal illness in any of the family members. Stool testing was not performed since the patient did not have clinical symptoms of gastroenteritis at presentation. Although the patient did not report a history of gastroenteritis, *Campylobacter* gastroenteritis can be subclinical. Neither the patient nor her family had handled raw or undercooked poultry. Upon further questioning, it was discovered that the family regularly drank unpasteurized cow's milk, which was likely the source of transmission.

At a follow-up appointment 1 week after discharge, a renal function panel was performed; all analytes were within normal limits except blood urea nitrogen (BUN), which was mildly elevated at 21 mg/dl (reference range, 7 to 20 mg/dl). Urinalysis revealed 3+ blood and 2+ protein but was otherwise normal.

DISCUSSION

The differential for curved Gram negative rods includes *Campylobacter* spp., *Helicobacter* spp., *Arcobacter* spp., *Anaerobiospirillum* spp., and *Vibrio* spp., although *Vibrio* spp. are comma-shaped rather than gull-shaped or helical. Some *Campylobacter* spp., such as *Campylobacter hominis*, form straight rods.

The genus *Campylobacter* includes at least 24 species. *C. jejuni* and *Campylobacter coli* are the most common species associated with human infections, but *Campylobacter fetus*, *Campylobacter lari*, and *Campylobacter upsaliensis*, among others, have also been reported to cause human infections (1). *Campylobacter* spp. stain poorly with Gram stain. The AO stain is a fluorescent stain that binds to nucleic acids of bacteria and cells. Bacteria appear bright orange, while human cells appear pale green to yellow (Fig. 1A). The AO stain has increased sensitivity compared to Gram stain and can aid in the diagnosis of true-positive blood cultures that are negative by Gram stain. However, the AO stain cannot be used as a replacement because it does not provide information about the Gram reaction. If the AO stain had not been performed in this case, the diagnosis would have been missed, as *C. jejuni* does not grow under routine incubation conditions. Although many labs have transitioned to molecular gastrointestinal testing, it is important to maintain appropriate incubation conditions for *Campylobacter*, as the genus is not restricted to gastrointestinal infections. An alternative to the Gram stain for the detection of *Campylobacter* spp. is to use carbol fuchsin rather than safranin for the counterstain. The substitution of carbol fuchsin makes it easier to visualize the organisms.

Campylobacter spp. are oxidase positive, are catalase variable, and exhibit motility on wet mount. *C. jejuni* is the most commonly isolated species of *Campylobacter* and can be differentiated from other species by its ability to hydrolyze hippurate. Of note, *Campylobacter avium* is also hippurate positive but is rarely encountered in clinical specimens. The two most common *Campylobacter* spp., *C. jejuni* and *C. coli*, grow well on *Campylobacter* selective media, but other species, such as *Campylobacter upsaliensis*, do not, hence the addition of chocolate agar when subculturing the positive blood culture broth. Selective media, such as CVA agar, are not necessary when subculturing positive blood culture broth since there is no normal flora for the organism to outcompete, as is the case with stool cultures. However, it may be useful if there is a second pathogen or a contaminant in the blood culture, and it may help narrow down the organism identification in labs that are not using MALDI-TOF MS. It is important to note that *C. fetus* is not thermotolerant and typically does not grow at 42°C. If only 42°C incubation is used, it may be missed.

The most common *Helicobacter* species isolated from blood, *Helicobacter cinaedi* and *Helicobacter heilmannii*, were less likely culprits based on the Gram stain. Both species are typically longer in length and are more helical. Anaerobic incubation at 35°C was added to attempt to isolate *Helicobacter*, *Anaerobiospirillum*, and possibly *Arcobacter* spp., if present. *Arcobacter* spp. grow microaerobically at 15 to 30°C, with variable growth at 35 to 42°C, and may grow aerobically at 30°C or anaerobically at 35°C. If the

lab is unable to isolate the organism on subculture, the positive blood culture broth can be sequenced to identify the organism.

The implementation of MALDI-TOF MS in our laboratory led to decreased turnaround times for *Campylobacter* species-level identification. The Biotyper claim 2 and claim 3 IVD libraries contain three *Campylobacter* species (*C. jejuni*, *C. coli*, and *C. ureolyticus*), and the research use only (RUO) library (version 7311) contains 19 species. In our laboratory, both the IVD and RUO libraries have been validated for use with clinical isolates. The IVD library is used for first-line identification, and unidentified isolates are tested using the RUO library. In our experience, non-*C. jejuni/C. coli/C. ureolyticus* isolates have given a result of "no identification" using the IVD library. In addition to no identification, an "unvalidated result" of the species has also been provided. These isolates were subsequently tested using the RUO library, and the results agreed with the unvalidated results.

Clinically, *Campylobacter* spp. most commonly cause gastroenteritis. The Centers for Disease Control and Prevention (CDC) estimates that *Campylobacter* infections affect more than 1.3 million people each year (https://www.cdc.gov/campylobacter/index.html). *Campylobacter* gastroenteritis can be diagnosed by stool culture using selective media, such as CVA agar, and microaerobic incubation conditions. However, the drawback of culture is that selective media are often selective only for *C. jejuni* and *C. coli* due to the presence of cefoperazone, a cephalosporin antibiotic. Multiplex molecular gastroenteritis panels, which often include *C. upsaliensis* or *C. lari* in addition to *C. jejuni* and *C. coli*, and antigen tests are becoming more common for the diagnosis of *Campylobacter* spp. due to their faster turnaround time and decreased labor requirements. Although they are less labor-intensive, antigen tests have suboptimal sensitivities and specificities (2).

C. jejuni has less commonly been associated with Guillain-Barre syndrome, hemolytic-uremic syndrome (HUS), and glomerulonephritis. The case reports of *C. jejuni* glomerulonephritis have been associated with preceding *Campylobacter* gastroenteritis (3). Poststreptococcal glomerulonephritis is the most common cause of infection-related glomerulonephritis, but other bacteria (e.g., *Bartonella* spp. and *Yersinia enterocolitica*), viruses (e.g., measles and mumps), fungi (e.g., *Coccidioides immitis/C. posadasii*), and parasites (e.g., *Plasmodium* spp. and *Toxoplasma gondii*) have also been associated with postinfectious glomerulonephritis. Acute glomerulonephritis is often a result of autoimmune diseases or infections. HSP (IgA vasculitis) is one of the autoimmune causes of glomerulonephritis. However, HSP was not considered a contributing factor in this case, as the patient had not had another episode since her HSP diagnosis 12 years prior.

Risk factors for campylobacteriosis include ingestion of raw or undercooked poultry and other foods contaminated by these items, ingestion of unpasteurized dairy products and untreated water, exposure to dog and cat feces, and immunocompromising conditions, most notably HIV/AIDS and hypogammaglobinemia. Immunocompromised hosts are at higher risk for developing invasive *Campylobacter* infections, especially bloodstream infections. The association of *Campylobacter* bacteremia is well reported in patients with hypogammaglobinemia (4).

The ingestion of unpasteurized dairy products is becoming more common due to the perceived health benefit of drinking raw milk. While it is illegal to sell or transport unpasteurized milk across state lines in the United States, some states have legalized the sale of raw milk directly from the dairy to the customer within their borders. In states where it is illegal to sell unpasteurized milk, dairies may sell shares of their dairy cows to the public or take other measures to bypass these laws. In addition to campylobacteriosis, ingestion of raw milk also increases the risk of infection from Shiga toxin-producing *Escherichia coli*, *Brucella* spp., *Listeria monocytogenes*, *Salmonella* spp., and *Cryptosporidium* spp.

Most patients with *Campylobacter* infection will recover without treatment. Treatment is indicated for patients with immunodeficiency and HIV infection. Treatment may be beneficial for patients with extraintestinal infection, persistent fevers, bloody stools, and symptoms lasting longer than a week. Treatment options for *C. jejuni* include azithro-

mycin and fluoroquinolones. However, resistance to ciprofloxacin is greater than 25% for *C. jejuni* and approaches 40% (39.8%) for *C. coli* (5). Azithromycin resistance ranges from 2.7% for *C. jejuni* to 12.7% for *C. coli*. Azithromycin interpretive criteria used in reference 5 were based on the epidemiological cutoffs (ECOFFs) established by the European Committee on Antimicrobial Susceptibility Testing (EUCAST). Multidrug-resistant isolates have been reported, with 4.4% of *C. jejuni* and 19.5% of *C. coli* isolates resistant to ≥3 classes of drugs (5).

SELF-ASSESSMENT QUESTIONS

1. Which of the following is a risk factor for campylobacteriosis?
    a. Camping
    b. Swimming in a chlorine-treated pool
    c. Ingestion of unpasteurized milk
    d. Ingestion of fried chicken
2. Most *Campylobacter* selective agars are selective only for which two species?
    a. *Campylobacter jejuni* and *Campylobacter coli*
    b. *Campylobacter coli* and *Campylobacter lari*
    c. *Campylobacter jejuni* and *Campylobacter lari*
    d. *Campylobacter jejuni* and *Campylobacter upsaliensis*
3. Which biochemical test can be used to differentiate *Campylobacter jejuni* from other clinically relevant *Campylobacter* species?
    a. Oxidase
    b. Catalase
    c. Motility
    d. Hippurate hydrolysis

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