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SHORT COMMUNICATION

Campylobacter jejuni bacteremia in a leukemic child: a nearly missed diagnosis

Ding, C.H.[1*], Wahab, A.A.[1], Tzar, M.N.[1], Mokhtar, M.N.[2], Arunasalam, V.[3]

[1]Department of Medical Microbiology and Immunology, Faculty of Medicine, Universiti Kebangsaan Malaysia, Kuala Lumpur, Malaysia
[2]Department of Anaesthesiology and Intensive Care, Faculty of Medicine, Universiti Kebangsaan Malaysia, Kuala Lumpur, Malaysia
[3]Department of Microbiology, Hospital Raja Permaisuri Bainun, Ministry of Health of Malaysia, Ipoh, Perak, Malaysia
*Corresponding author: dingch@ppukm.ukm.edu.my

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ABSTRACT

Globally, *Campylobacter* spp. are responsible for most cases of bacterial gastrointestinal infections in humans and although rare, extraintestinal *Campylobacter* infections have been described. A 2-year-old neutropenic girl with underlying precursor B-cell acute lymphoblastic leukemia presented with a 3-day history of diarrhea. Her stool culture yielded no enteric bacterial pathogens. However, when her blood culture was flagged as positive for bacterial growth, no colonies could be observed on routine bacteriological isolation media. Nonetheless, gram-negative bacilli with seagull and spiral morphologies were seen when the surface of the isolation media used to subculture her blood was Gram-stained. Bacterial colonies were only visible when a subculture was attempted on a *Campylobacter* blood-free selective agar medium. The organism was identified as *Campylobacter jejuni* by matrix-assisted laser desorption ionization-time of flight mass spectrometry. Since the organism was erythromycin-resistant and the patient's age precluded the use of tetracycline and ciprofloxacin, an antibiotic regimen consisting of piperacillin-tazobactam and gentamicin was commenced. Her *C. jejuni* bacteremia resolved following eight days of antibiotic therapy.

Keywords: Bacteremia; *Campylobacter jejuni*; campylobacteriosis; leukemia; neutropenia.

INTRODUCTION

Campylobacter jejuni is a zoonotic pathogen with numerous warm-blooded animal reservoirs, of which poultry, wild birds, cattle, swine and sheep are notable examples (Mughal, 2018). Accordingly, *C. jejuni* can be transmitted to humans when contaminated food or water (e.g., inadequately cooked meats, unboiled water or unpasteurized milk) is ingested, or when there is direct contact with fecal material from infected animals or humans (Sahilah *et al*., 2010). Although *Campylobacter* infections are often self-limiting and entail only supportive therapy, specific antimicrobials are warranted in patients who are either immunocompromised or have extraintestinal campylobacteriosis (such as bacteremia) (Zenebe *et al*., 2020). Alas, the rampant use of antimicrobials such as tetracycline and ciprofloxacin in livestock farming as growth promoters has orchestrated the emergence of resistance in *Campylobacter* spp. to the very same drugs employed to treat campylobacteriosis (Premarathne *et al*., 2017). We report a case of antibiotic-resistant *C. jejuni* bacteremia in a leukemic child who was rendered neutropenic as a result of chemotherapy.

CASE REPORT

A 2-year-old girl with underlying precursor B-cell acute lymphoblastic leukemia presented to Hospital Raja Permaisuri Bainun with a 3-day history of diarrhea. She had just completed her second cycle of cytotoxic chemotherapy (consisting of vincristine, L-asparaginase, doxorubicin and dexamethasone) a day prior to the onset of diarrhea. The diarrheal stool was mixed with mucus and occurred up to 15 times a day. Although oral intake was reduced, there was no vomiting. There was no recent history of eating hawker or restaurant food. Neither the patient nor her family members consume improperly cooked meats, unboiled water or raw milk. On examination, the girl was lethargic, had cold peripheries and a weak pulse volume. She had a blood pressure of 105/71 mm Hg, a pulse rate of 110 beats/minute, a temperature of 36.6°C and a room air oxygen saturation of 99%. Cardiovascular, respiratory and abdominal examinations were unremarkable. However, blood investigations revealed a reduced total white cell count of only 0.1 x 10^9 cells/L (the neutrophil and lymphocyte counts were 0.03 x 10^9 cells/L and 0.02 x 10^9 cells/L, respectively), a low hemoglobin level of 6.4 g/dL, a low platelet count of 67 x 10^9 cells/L and an elevated C-reactive protein level of 72.8 mg/dL. Dehydration was evident from her renal profile, with serum urea and creatinine levels of 6 mmol/L and 29 umol/L, respectively.

A diagnosis of neutropenic sepsis was made, and blood as well as stool specimens were taken for microbiological investigations. In the meantime, empirical antibiotic therapy consisting of intravenous piperacillin-tazobactam and gentamicin was promptly commenced. Rehydration was achieved by administering half of her fluid maintenance needs intravenously and providing oral rehydration salts via a Ryle's tube. Oral intake was encouraged, although milk feeds were withheld. Her stool was negative for rotavirus antigen, as well as for parasitic ova and cysts. No enteric bacterial pathogen

was isolated following a stool culture. On day 3 of incubation, her blood culture vial was flagged as positive by the automated blood culture system (BD BACTEC™ FX). However, no organism could be seen when the vial's content was Gram-stained. Nonetheless, the vial's content was still inoculated onto various primary isolation media (i.e., sheep blood, Sabouraud dextrose, MacConkey and chocolate agars).

After an overnight incubation, no bacterial colonies were visualized on any of our agar plates. Notwithstanding this, the first quadrant of the blood agar used to streak the inoculum was subjected to a perfunctory Gram stain. Astonishingly, gram-negative bacilli with occasional seagull and spiral morphologies were seen (Figure 1). Suspecting *Campylobacter* sp., we proceeded to subculture the organism on a *Campylobacter* blood-free selective agar medium (CM0739, Oxoid Ltd, UK) supplemented with cefoperazone and amphotericin B (SR0155, Oxoid Ltd, UK). The plate was incubated at 37°C in a microaerophilic (~5% O_2) atmosphere. After 48 hours, small (up to 3 mm in diameter) and flat gray colonies were seen (Figure 2). The isolate's identity was confirmed as *C. jejuni* by matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI Biotyper, Bruker-Daltonics, Germany), when its mass spectral pattern matched that of *C. jejuni* MB_6111_05 THL with a score value of 2.32.

Antibiotic susceptibility testing for *C. jejuni* was performed as recommended by the Clinical and Laboratory Standards Institute (CLSI) in its M45 document (CLSI, 2016). Through the disk diffusion method, the isolate was found to be resistant to erythromycin. Tetracycline and ciprofloxacin, which are also recommended by the CLSI for primary testing, were not tested given that these antibiotics are generally contraindicated in young children. Faced with a dearth of laboratory-sanctioned treatment options, the pediatric team decided to continue administering piperacillin-tazobactam and gentamicin for a total duration of eight days. The patient was well and discharged home following the completion of her antibiotic therapy and after a repeat blood culture confirmed the bacteriological clearance of *C. jejuni*.

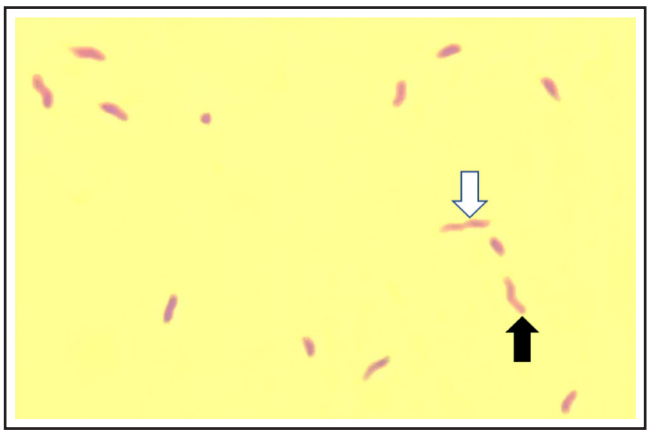

Figure 1. Microscopic examination of the organism at 100x magnification revealed gram-negative bacilli with seagull (black arrow) and spiral morphologies (white arrow).

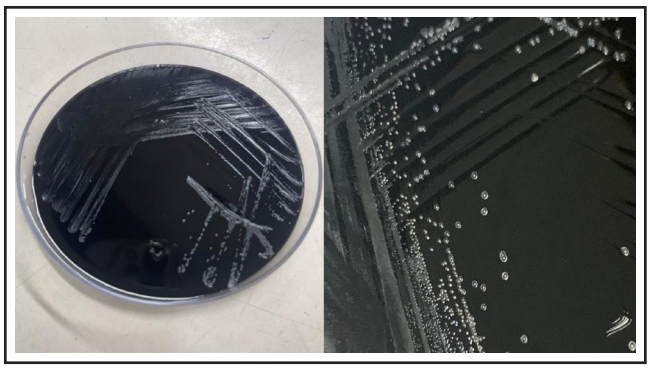

Figure 2. The organism grew as small and flat gray colonies on a *Campylobacter* blood-free selective agar plate after 48 hours.

DISCUSSION

Although the *Campylobacter* genus was proposed six decades ago, the first reported *Campylobacter* case was believed to date back more than a century ago to 1886, when "spiral-shaped bacteria" were found in the colons of children with an enteric disease known as "cholera infantum" (Epps *et al*., 2013). In fact, due to their uncanny morphological resemblance to vibrios, *C. jejuni* and *C. coli* were once called *Vibrio jejuni* and *V. coli*, respectively (Epps *et al*., 2013). Among the bacterial characteristics instrumental in establishing the *Campylobacter* genus is the microaerophillic (defined as an oxygen concentration of 3-15%) growth requirement of its members (Kim *et al*., 2015). Today, despite the *Campylobacter* genus containing at least 39 species with 16 subspecies, nearly 90% of reported *Campylobacter* cases are attributed to *C. jejuni* alone (Fitzgerald, 2015; Zenebe *et al*., 2020). This observation is not merely of academic interest, as *C. jejuni* is the species most implicated as the cause of Guillain-Barr Syndrome, an autoimmune disease inextricably linked to the nervous system (Kaakoush *et al*., 2015). *C. jejuni* is also one of the species that has been well-documented in the medical literature to cause septicemia in humans (Fitzgerald, 2015).

This case is perplexing not because of the rarity of *Campylobacter* gastroenteritis in children. Rather, what puzzled us was the absence of an unequivocal risk factor from the clinical history suggesting a route of transmission for the organism. Thus, even though our patient was admittedly neutropenic (which by itself is already a risk factor for a myriad of infections), we did not consider campylobacteriosis at the outset – due to its low infectious dose (postulated to be as low as 360 CFU), *C. jejuni* also infects many immunocompetent individuals (Kaakoush *et al*., 2015). By and large, *Campylobacter* gastroenteritis in children is not clinically distinguishable from other bacterial or even viral causes of gastroenteritis (Same & Tamma, 2018). Judging from the age of the patient, we were initially more inclined to consider a rotaviral diarrhea. The inability to isolate *Campylobacter* sp. in the stool culture despite using a selective *Campylobacter* agar further served as a "red herring" in the early stages of our microbiological investigation. However, the sensitivity of stool culture in diagnosing campylobacteriosis can be as low as 60% due to diverse factors such as the erratic death of *Campylobacter* bacilli during specimen handling and the difficulty in spotting tiny *Campylobacter* colonies amongst competing fecal flora (Buss *et al*., 2019).

Apart from its typical morphology and microaerophilic culture requirement, *C. jejuni* is known to have a narrow temperature range for its optimal growth, which is between 37-42°C (Davis & DiRita, 2008). An assortment of blood- or charcoal-based agar media are available commercially for the isolation of *C. jejuni* from human specimens (Fitzgerald, 2015). The medium we utilised was a blood-free modified charcoal cefoperazone deoxycholate agar (mCCDA). A comparison between this agar and two blood-containing agars (i.e. Skirrows and Preston agars) showed that mCCDA was more sensitive in detecting *Campylobacter* spp. (Rodgers *et al*., 2012). Some authors propose an incubation temperature of 42°C when culturing *C. jejuni* (Kaakoush *et al*., 2015). However, we incubated our isolate at 37°C on the agar manufacturer's recommendation, which in turn was based on the discovery that more campylobacters could be isolated successfully on mCCDA when incubated at 37°C instead of 42°C (Bolton *et al*., 1988). This is because unlike *C. jejuni* and *C. coli*, there are non-thermotolerant campylobacters (e.g., *Campylobacter concisus* and *Campylobacter upsaliensis*) that are

likely to be missed if incubating at a higher temperature is routinely practiced.

The treatment of campylobacteriosis is hardly straightforward, due to both intrinsic and acquired antibiotic resistance issues. Specifically, *C. jejuni* is intrinsically resistant to polymyxins, which are habitually relied on to treat infections caused by highly-resistant gram-negative bacteria (Iovine, 2013). In Asia, acquired *C. jejuni* resistance to fluoroquinolones is alarming, with Thailand reporting resistance rates in excess of 80% (Luangtongkum *et al*., 2009). The published tetracycline resistance rates are not comforting either, with Taiwanese investigators reporting a jaw-dropping resistance rate of 95% in their *C. jejuni* isolates (Li *et al*., 1998). Both the Thai and Taiwanese studies were conducted at least a decade ago – it is therefore plausible that the resistance rates to both antibiotics have since escalated. To complicate matters, these two antibiotics are relatively contraindicated in the pediatric population, further contracting the pool of available antibiotics to treat campylobacteriosis in certain situations. Macrolides appear to fair better against *C. jejuni*, with reported erythromycin resistance rates ranging from 0-11% (CLSI, 2016). Unfortunately, since our isolate was erythromycin-resistant we had to resort to atypical and non-time-tested antibiotics for treatment, such as piperacillin-tazobactam. While there is a paucity of success stories in the medical literature on the use of piperacillin-tazobactam against *C. jejuni*, a case report from Brazil chronicled the successful treatment of *Campylobacter fetus* bacteremia with piperacillin-tazobactam (Coustillɒres *et al*., 2022).

CONCLUSION

C. jejuni bacteremia should be considered when a neutropenic patient presents with acute gastroenteritis symptoms. Effective communication between the clinician and the laboratorian is mandatory because the organism may be missed on routine cultures sans a high index of suspicion. Utilizing selective campylobacter media such as mCCDA to culture blood in such cases is warranted. Antibiotic susceptibility testing of *C. jejuni* is also obligatory due to its pervasive antibiotic resistance issues.

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Conflict of Interest

The authors declare that they have no conflicts of interest.

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