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Original Article

First finding of Ciprofloxacin Resistant *Campylobacter jejuni* Bacteremia from an infant with biliary atresia in Surabaya, Indonesia

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Abstract
Here we present a case of an infant with biliary atresia with bacteremia caused by Ciprofloxacin resistant *C. jejuni*. An eight-month-old boy - previously diagnosed with inoperable biliary atresia, was referred with fever. The isolated bacteria was identified as *Campylobacter jejuni ssp. jejuni*. This isolate was resistant to Ciprofloxacin using disk diffusion. From our investigation during the patient's home visit, there were several chicken and bird coops within 20 meters radius all around the patient's house, contributing to the risk of infection. In this case, a severe infection of *Campylobacter jejuni* might be promoted by biliary atresia and hepatic failure.
***Keywords**: Campylobacter jejuni*, Bacteremia, Biliary atresia, Ciprofloxacin resistant

Introduction

Campylobacter jejuni is one of the causes of zoonotic and foodborne infections. It is introduced to humans by contamination of poultry products, domestic birds, dairy products, and ingested materials contaminated with bacteria. In 2017, the Centers for Disease Control and Prevention reported *Campylobacter* as the highest cause among all foodborne infections (Marder et al., 2018). Although studies done in Asia have shown that *C. jejuni* is the predominant species in human infections (Liu et al., 2022), and *C. jejuni* bacteremia cases are well-known, but they are still rarely found. Literature has mentioned Human Immunodeficiency Virus infection, malignancy, liver cirrhosis, and malnutrition as underlying conditions associated with *C. jejuni* bacteremia (O'hara et al., 2017; Karama et al., 2020; Moffatt et al., 2021; Mizuno et al., 2022), but only a few studies have reported biliary atresia as an underlying condition associated with *Campylobacter spp.* bacteremia (Mizuno et al., 2022). Around one-third of *C. jejuni* isolates in South Africa were reported as Multi-Drug Resistant *C. jejuni*. Most of the isolates were reported to be resistant to clindamycin, nalidixic

acid, tetracycline, and erythromycin (Karama et al., 2020).

Case Report

An eight-month-old boy was referred to a referral public hospital with a fever and inoperable biliary atresia. The fever occurred a week before admission. The jaundice was noted since two weeks of age. Ascites was prominent two months before admission. Gastrointestinal complains were denied. The patient was breastfed until two months of age and formula-fed thereafter. Weaning started at six months of age, and developmental milestones were suitable for his age. His family did not have similar complaints.

Upon examination, the patient was lethargic, had greenish jaundice, 38.5°C fever, hepatomegaly, massive ascites, and was in severe wasting condition. Laboratory studies showed microcytic-hypochromic anemia (MCV 83 fL, MCH 26 pg), leukocytosis (27.390 cells/µL), hyperbilirubinemia (12.16 mg/dL), direct hyperbilirubinemia (7.8 mg/dL), and aspartate aminotransferase and alanine aminotransferase levels were 103 and 47 U/L, respectively. The alkaline phosphatase level was 8.33 mg/dL, and hypoalbuminemia (1.4 mg/dL), prolonged prothrombin time, and activated partial prothrombin time were also observed. Systemic bacterial infection was confirmed by a high procalcitonin level (29.49 ng/mL). Biliary atresia was confirmed with a triangular cord sign and hepatic subcapsular flow from abdominal ultrasound (Figure 1A).

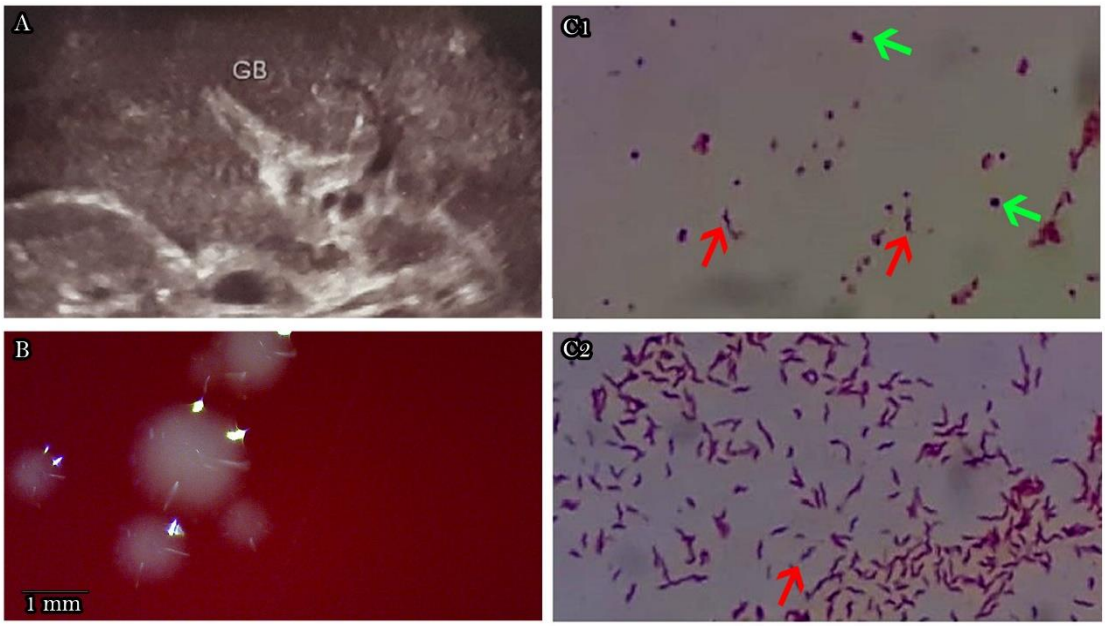

Fig. 1. Abdominal ultrasonography showing triangular cord sign confirming biliary atresia (A). (B) *Campylobacter jejuni* colony appearance on Skirrow media. The colony size is small to moderate, mucoid, round shape, and grey pigmentation with a glistening surface. Direct Gram staining of Campylobacter from blood culture showed a predominance of coccobacilli forms (green arrows) with several seagull appearances (red arrows) (C1) while staining from colonies incubated from Skirrow's medium exhibited longer spirals and seagull appearances homogenously (C2).

The patient was given intravenous ceftriaxone 450 mg, b.i.d for the first seven days, but no clinical improvement was observed, which urged the pediatrician to request a blood culture. Before the blood culture result, the antibiotic was replaced with intravenous cefoperazone-sulbactam 400 mg,

b.i.d for three days, then with intravenous meropenem 200 mg, t.i.d for seven days. Other than antibiotics, intravenous albumin correction was also performed.

A blood culture sample was collected after seven days of hospitalization and detected positive after 47 hours of incubation at 35°C with an aerobic BACTEC® BD® blood culture system. Direct Gram preparation from BACTEC® pediatric blood culture exhibited Gram-negative coccobacilli with a little number of seagull-appearance morphologies, after incubation under the microaerophilic conditions in a candle jar for 48 hours at 35°C. Gram stain for the colony from Skirrow media showed Gram-negative spirals with more prominent seagull-appearance morphology. The isolated organism was oxidase and catalase positive. It was identified as *Campylobacter jejuni ssp. jejuni* with 97% probability using VITEK 2® NH® ID card (Biomerieux™). The antimicrobial susceptibility test by disk diffusion method showed no inhibition zone for the ciprofloxacin 5 µg disk. The inhibition zone diameter around the erythromycin 5 µg was 20 mm. Susceptibility to tetracycline was not tested due to the unavailability of the disk in Indonesia at that time. The total turnaround time for the blood culture was seven days after the specimen was collected.

The patient showed significant clinical improvements and was sent home after fifteen days of hospitalization. He came back for a routine post-discharge check-up one week after with no sign of infection, and improvement in ascites, but worse jaundice was observed. We performed a house visit two weeks after hospital discharge. The general condition of the patient was good – other than the massive ascites and jaundice. The house was well situated in a rural housing complex common in Indonesia. The house was clean and tidy. The family drink water from branded water gallons.

There were several chicken and bird coops within 20 meters radius all around the patient's house. The neighbors were keeping birds in cages. We found that the disposal tray of the cages that belong to the neighbor across the patient's house was filled with fecal materials. There was scattered rodent fecal material that was observed in front of the house. With the patient's family and neighbor's consent, culture samples were obtained from water waste, drinking water sources, bird coops, rodents, and poultry litter. No Campylobacter was found microscopically or by cultivation using Skirrow media in microaerophilic conditions. Unfortunately, the patient died after severe variceal bleeding five weeks after hospital discharge.

Discussion

The microscopic morphology of *C. jejuni* observed directly from the aerobic blood culture was Gram-negative coccobacilli with only a small number of seagull appearances. This morphology shift suggested that the organism was probably going through acute stress, which in turn might contribute to a higher level of virulence (Pokhrel et al., 2022). The morphology changed to the typical Gram-negative long spiral under microaerophilic conditions (Figures 1C and 1D). The morphological differences in this isolate might describe the reduction or promotion of certain virulence factors such as metabolism, unipolar flagella, and growth speed. This characteristic might be elicited by previous stressors such as antibiotics or fever. This feature also appeared in *Streptococcus pneumoniae*, *Escherichia coli,* and *Helicobacter pylori* (Cushnie, 2016).

The antimicrobial susceptibility tests, in this case, were performed using disk diffusion test as per CLSI M45-A2 standard procedures and breakpoints. The limitation in the interpretation of the guideline is that the susceptibility cannot be interpreted, while resistance can be concluded if there is no inhibition zone. The dilution method provides a better interpretation of the test. Meanwhile, CLSI document M45-3 has stated that the susceptibility of Campylobacter could be interpreted from the disk diffusion method. With this update, erythromycin, in this case, can be interpreted as susceptible (CLSI, 2015).

A research study in Tokyo has found that out of 21 cases of Campylobacter bacteremia, the median time to positivity of blood culture using BACTEC®, was 55.3 hours (50.3–67.6 hours).

The research stated that 89% of their patients were given beta-lactam or carbapenem before the blood culture was taken (Yamamoto et al., 2017). The prolonged lag phase in this patient is probably attributed to previous stressors such as antibiotics.

Gut microbiota metabolizes the antimicrobial bile acids through the process of deconjugation. This patient fails to secrete bile salt due to atresia; therefore, microbial defense is compromised. Disturbances of intestinal pH levels and ascites also contribute to tight junction impairments and increase the vulnerability to exogenous invasion, in this case, *C. jejuni* (Raja et al., 2018). In an acute infection, the liver controls homeostatic inflammatory processes such as acute phase reactants (antimicrobial proteins and cytokines, opsonin and leukocyte mobility, coagulation and complement factors), vasoactive agents, and tolerance towards LPS. In biliary stasis and cirrhosis, the liver fails to give an adequate defensive response; therefore contributes to the invasiveness of *C. jejuni* (Robinson et al., 2016). Massive ascites and portal hypertension alters intestinal permeability to toxins and microorganisms and promote pathogenic invasion. In other cases, the pathogenic invasion might result in spontaneous bacterial peritonitis (Zhang and Faust, 2021). The accumulated impairment of host and microbial defense of the intestinal mucosa due to the inoperable biliary atresia contributes to the invasiveness of *C. jejuni.* Although Campylobacter screening from the environment of the house resulted in negative, Campylobacter transmission from poultry around the house is still plausible. Dried fecal material can be blown toward the house. Campylobacter can also form a viable but non-culturable state, lowering sensitivity to environmental isolation (Pokhrel et al., 2022).

The results of the studies have indicated that the rising use of antibiotics such as fluoroquinolones and tetracycline contributes to the emergence of resistant *Campylobacter* due to efflux pump and other mechanisms (Yao et al., 2016; Sproston et al., 2018; CDC, 2019; Mak et al., 2020). The Indonesian government has regulated antimicrobial use within veterinary practices in Indonesia. The use of antimicrobial and hormonal agents as additives in cattle food and the use of antibiotics as growth promoters inside registered commercial cattle food is banned (Government of The Republic of Indonesia, 2014; Ministry of Agriculture, 2017). Even so, reports of ease of access to antibiotics for animals are still evident. According to the authors observations, some combinations of tetracycline and chloramphenicol, oxytetracycline, terramycin, and neomycin for poultry and fish are available to be accessed through some online shopping platforms. Some online shops can provide repacked powders and allow transactions without a prescription. Human access and the use of antibiotics in Indonesia has been restricted since 2020. The government has regulated the availability and types of antibiotics that are sold in pharmacies. The regulation stated that antibiotics used by humans can only be purchased with prescription and be sold under registered pharmacist (Ministry of Health, 2020).

Conclusion

This case adds to the little pieces of literature published about ciprofloxacin-resistant *C. jejuni* bacteremia in which contact with poultry and biliary atresia act as the possible risk factors.

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Conflict of interest statement

The authors have no conflicts of interest to declare.

Ethical approval

The written and verbal consent for the publication of this case have been obtained from the parents. Information related to identity has been omitted from the case presentation.

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