Campylobacter jejuni bacteremia in Italian pediatric patients with acute lymphoblastic leukemia: Report of two cases.

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SUMMARY

Infections caused by *Campylobacter jejuni* are rarely associated with extraintestinal complications. *C. jejuni* bacteremia is difficult to detect in patients with hematological malignancies undergoing chemotherapy where the choice of appropriate antibiotic treatment is extremely important. We report two cases of *C. jejuni* bacteremia in Italian pediatric patients affected by acute lymphoblastic leukemia (ALL). Agreeing with the most recent epidemiological data, both clinical isolates showed a typical phenotypic antimicrobial resistance patterns with combined resistance to ciprofloxacin and tetracycline.
To our knowledge, this is the first report of *C. jejuni* isolation from the blood of ALL pediatric patients in Italy, and it provides important epidemiological information on this rare infection.

INTRODUCTION

Campylobacteriosis is an infectious disease caused by the Gram-negative, spirally curved, non-spore forming rods *Campylobacter* bacterium. In 1972 it was recognized as an etiologic agent of gastroenteritis in humans (Chlebicz *et al.*, 2018).
Campylobacteriosis is considered one of the most frequent causes of foodborne infection and one of the main public health problems in Europe and worldwide (EFSA 2015, Silva *et al.*, 2011). The natural reservoirs of *Campylobacter* species are intestinal tracts of birds and mammals. Eating or handling contaminated/undercooked meat, especially poultry, are considered to be major risk factors for human campylobacteriosis. *Campylobacter* enteritis is the most frequent form of acute bacterial diarrhea in industrialized countries (Portner *et al.*, 2007) especially in children and young adults. The species most commonly associated with human infection are *C. jejuni*, *C. coli* and *C. fetus* (Butzler 2004). They differ from other pathogenic bacteria transmitted by food as they grow in an atmosphere containing nearly 10% CO_2 and 5% O_2, but some species can also grow aerobically or anaerobically, in a narrow range of temperatures, from 30 to 46°C. *Campylobacter* species have a tendency to form viable but nonculturable cells in hostile growth environments, and therefore can be undetected or underestimated by culture methods (EFSA 2012).

Unlike other enteric infections, campylobacteriosis is only rarely associated with extraintestinal complications and systemic invasive illness (Pacanowski *et al.*, 2008).
In Europe, bacteremia due to *Campylobacter* spp has been detected in less than 1% of the patients with gastroenteritis and has been reported in immunocompromised patients and in young or in elderly patients (Fernandez-Cruz *et al.*, 2010). The low detection rate could be partly explained by under diagnosis due to bactericidal properties of human serum against the species (Gallo *et al.*, 2016). Another possible explanation is that blood cultures are not routinely performed for acute gastroenteritis, even when patients are febrile (Gallo *et al.*, 2016) or show the typical symptoms (cytopenia, diarrhea, etc.) undergoing chemotherapy (Pacanowski *et al.*, 2008).
The aim of this study was to report two clinical cases of pediatric patients with acute lymphocytic leukemia (ALL) who were infected with *C. jejuni*. To the best of our knowledge, this is the first report of *C. jejuni* isolation from the blood of ALL patients in Italy.

CASE REPORT

Patient 1

We describe the medical records of a pediatric patient, with diagnosis of ALL, who had bacteremia caused by *C. jejuni*. In June 2014, a 4-year-old female visited the U.O. of Pediatric Oncology of University of Campania "Luigi Vanvitelli" and presented night sweats, severe polar, onset of diarrhea, and fever. Previously diagnosed with ALL at
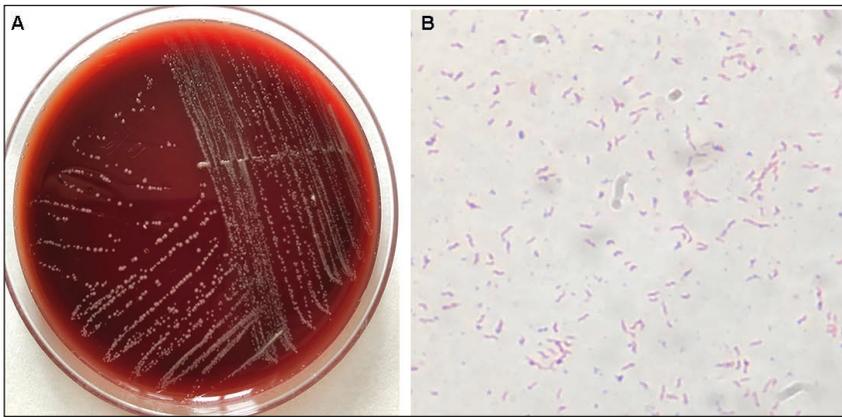
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Figure 1 - *Macroscopic and microscopic appearance of* C. jejuni. *(A) Macroscopic appearance of* C. jejuni *colonies following isolation from patient and culture on blood agar plates incubated in microaerophilic conditions for 72hr at 37°C. (B) Gram stain from pure culture of* C. jejuni *cultured on blood agar at 37°C for 24hr.*

intermediate risk, the patient had received chemotherapy. On admission, her temperature was 37.7°C, heart rate was 110 beats/minute, and respiratory rate was 33 breaths/minute. The complete blood count included hemoglobin (Hb) of 10.5 g/dl, white blood cell count of 1610/mm³, with 515/mm³ neutrophils, 998/mm³ lymphocytes; platelet count of 244,000/mm³. Other results of laboratory tests were: total bilirubin, 1.21 mg/dl; direct bilirubin, 0.43 mg/dl; aspartate transaminase, 39 IU/L; alanine transaminase ALT, 22 IU/L; total protein, 4.1 g/dl; albumin, 3.1 g/dl; lactate dehydrogenase, 666 U/L; Erythrocyte Sedimentation Rate (ESR), 22 mm and serum C-reactive protein (PCR) level, 4.7 mg/dl. Simultaneously, an aerobic blood culture from central venous catheter was performed using an automated blood cultivator, BACTEC 9240 system (Becton Dickinson), and this culture was positive after 24 hours. Gram staining of the BACTEC tube showed curved gram-negative motile rods (*Figure 1B*). Subcultures were performed on culture media, including Columbia CNA Agar with 5% Sheep Blood, MacConkey agar, Chocolate agar and Sabouraud agar (BioMérieux) (Iovene et al., 2018a; Iovene et al., 2018b; Iaffaldano et al., 2018). Unfortunately, no microorganism growth on culture media was observed after incubation.

Since we suspected the presence of an anaerobic or microaerophilic microorganism, we performed an anaerobic and microaerophilic blood culture, which was positive after 48 hours within 35°C to 37°C. Gram staining from the blood culture revealed slightly curved gram-negative bacilli. After that, the blood sample was spread on Columbia agar enriched with yeast extract, fetal calf serum and horse blood, under anaerobic and microaerophilic conditions for 72 hours at 37°C (*Figure 1A*). The patient was also positive to *C. jejuni* fecal antigen.

Table 1 - *Antibiotic susceptibility profile of C. jejuni clinical isolates.*

Antimicrobial agents	C. jejuni (Patient 1)	C. jejuni (Patient 2)
Ciprofloxacin	resistant	resistant
Azithromycin	susceptible	susceptible
Clarithromycin	susceptible	susceptible
Erythromycin	susceptible	susceptible
Doxycycline	resistant	resistant
Tetracycline	resistant	resistant

Parallelly, the patient underwent empiric therapy first with ceftriaxone (for three days) and then with sulfamethoxazole/trimethoprim and amoxicillin/clavulanic acid. After three days, the patient suspended therapy with amoxicillin/clavulanic acid, and shortly thereafter began a new cycle of chemotherapy. The following day the fever reappeared and aerobic and anaerobic blood cultures from central venous catheter were performed. The primary culture was positive for *Campylobacter*, and the subculture from the solid plate yielded a confluent growth of colonies typical for *Campylobacter*. The bacterial identification using Vitek 2 system (BioMérieux) indicated *C. jejuni subsp. jejuni* (99%) (Iovene et al., 2018a; Iovene et al., 2018b; Ferrazzano et al., 2017). The isolate *C. jejuni* was tested for antimicrobial susceptibility using Vitek 2 system (*Table 1*) and exhibited resistance to ciprofloxacin, doxycycline and tetracycline. According to the obtained results, the patient began antimicrobial therapy with clarithromycin for 10 days.

Patient 2

A 7-year-old boy with ALL (high risk), diagnosed one year before the present admission. In October 2015, this child visited the same Pediatric Oncology district with bouts of diarrhea, reduced appetite and nausea, followed by febrile episodes in the following days. His temperature was 38.7°C, heart rate was 120 beats/minute, and respiratory rate was 36 breaths/minute. Laboratory examination revealed Hb of 10.6 g/dl, a decreased white blood cell count of 1260/mm³, with 731/mm³ neutrophils and 409/mm³ lymphocytes and platelet count of 147,000/mm³. Normal coagulation. Other blood tests were normal, except for ESR, 93 mm and PCR, 10 mg /dl.

After two consecutive blood cultures taken from the peripheral vein and central venous catheter line, cefixoral was administered as empiric therapy. The blood cultures exhibited only gram-negative from the aerobic culture less than 24 hours of incubation. The patient was also positive for *C. jejuni* faecal antigen. The bacterial identification using Vitek 2 system indicated *C. jejuni subsp. jejuni* (95%). Antimicrobial susceptibility test of the isolated strain obtained using Vitek 2 system was reported in Table 1. The *C. jejuni* isolate exhibited resistance to ciprofloxacin, tetracycline and doxycycline. The combined antimicrobial therapy with amoxicillin/clavulanic acid and sulfamethoxazole/trimethoprim finally led to a significant clinical improvement in symptoms. Antimicrobial treatment was administered for 15 days until the patient's neutropenia and clinical symptoms were improved.

DISCUSSION

C. jejuni infections are typically self-limited in immunocompetent subjects, and severe extra intestinal complications are uncommon. However, a reduced immune system response, which may occur in elderly people, immunocompromised hosts or patients with debilitating conditions increases the risk of developing a severe infection from this pathogen (Pacanowski *et al.,* 2008; Fernanadez-Cruz *et al.,* 2010; Nielsen *et al.,* 2010). In the present clinical case report, we describe two clinical cases of bacteremia caused by *C. jejuni* in children affected by ALL in Italy.

In many hematological malignancies, especially lymphoid neoplasms, and in patients undergoing chemotherapy, the early recognition of *C. jejuni* bacteremia symptoms may be crucial to start the most adequate treatment.

Furthermore, the impact of appropriateness of treatment on mortality is debated. Although Pacanowsky et al demonstrated that failure of a prompt appropriate antimicrobial therapy in *C. jejuni* bacteremia was associated with fatal outcome (Pacanowski *et al.,* 2008), some recent retrospective studies reported that antimicrobial treatment did not affect the outcome of infection to any great extent (Liao *et al., 2012;* Feodoroff *et al.,* 2012).

Thus, from our experience and from the literature (Pacanowski *et al.,* 2008; Nielsen *et al.,* 2010), we propose that blood culture should always be performed in febrile patients with acute gastroenteritis illnesses, especially when the severity of the infection could be amplified by predisposing clinical conditions (Mearelli *et al.,* 2017). In addition, in case of positive BACTEC results in aerobic conditions with no possibility to isolate microorganism (Patient 1), it is strongly recommended to carry out an anaerobic culture, especially for immunocompromised patients. Based on our observations, we highlight the importance of the clinician's alert to the clinical microbiology laboratory to the possibility of *C. jejuni* infection when there is a compatible clinical syndrome, so that appropriate culture media and incubation conditions are used.

The emergence of this bacterial type as responsible agent of infections in a human host and its tendency to carry antibiotic resistance elements leads to a failure of chemoprophylaxis, thus making it a serious threat to public health.

Different strategies were adopted for the empirical treatment of *C. jejuni* infection, including fluoroquinolones (e.g., ciprofloxacin), macrolides (erythromycin), and aminoglycosides (gentamicin) (Blaser *et al.,* 2008).

Recent literature data report high rates of ciprofloxacin resistance (>50%) in both environmental and clinical isolates (Szczepanska *et al.,* 2017). According to a recent epidemiological report (Liao *et al.,* 2012; Mearelli *et al.,* 2017; Fernández-Cruz *et al.,* 2010), the clinical strains recovered in our work showed antimicrobial resistance patterns with combined resistance to ciprofloxacin and tetracycline. Both the excessive use of these antimicrobial agent in clinical practice and the employment of fluoroquinolones in food-producing animals and in veterinary species represent the cause of the increase of resistant strains. Thus, the possibility of fluoroquinolone-resistant strains must be considered in all cases of *Campylobacter* bacteremia (Gallo *et al.,* 2016).

In conclusion, this is the first report of *C. jejuni* bacteremia in immunocompromised pediatric patients with ALL in Italy. It provides important epidemiological information on this rare infection, showing that both clinical isolates presented typical phenotypic antimicrobial resistance patterns with combined resistance to ciprofloxacin and tetracycline. Moreover, additional studies are required to define the pathogenicity and clinical aspect of *C. jejuni* bacteremia as well as those of other *Campylobacter* spp.

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