

## A ten-year review of antimicrobial resistance among human *Campylobacter jejuni* isolates

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1. NRL Enteric diseases, pathogenic cocci and diphtheria, NCIPD

### Summary

*Campylobacter jejuni* is a major pathogen responsible for a significant number of reported campylobacteriosis cases in the European Union. Due to underdiagnosed and underreported cases, there are no existing studies on this pathogen. Fluoroquinolones have been the primary treatment for campylobacteriosis for many years. Fluoroquinolone resistance is causing an increase, which means that a new class of antibiotics must be introduced. The treatment of campylobacteriosis is currently based on macrolides, but with the increasing rates of resistance, active surveillance for antibiotic resistance is required. The aim of this study was to track the increasing prevalence of AMR in clinical isolates of *C. jejuni* across Bulgaria for a period of 10 years.

**Keywords:** *Campylobacter jejuni*, Antimicrobial resistance (AMR), Ciprofloxacin, Erythromycin.

### Introduction

*Campylobacter jejuni* is one of the most common causative agents of acute bacterial gastroenteritis worldwide and in our country. In recent years, antimicrobial resistance (AMR) of the species has risen sharply, according to the ECDC and EFSA. The focus of this study was to

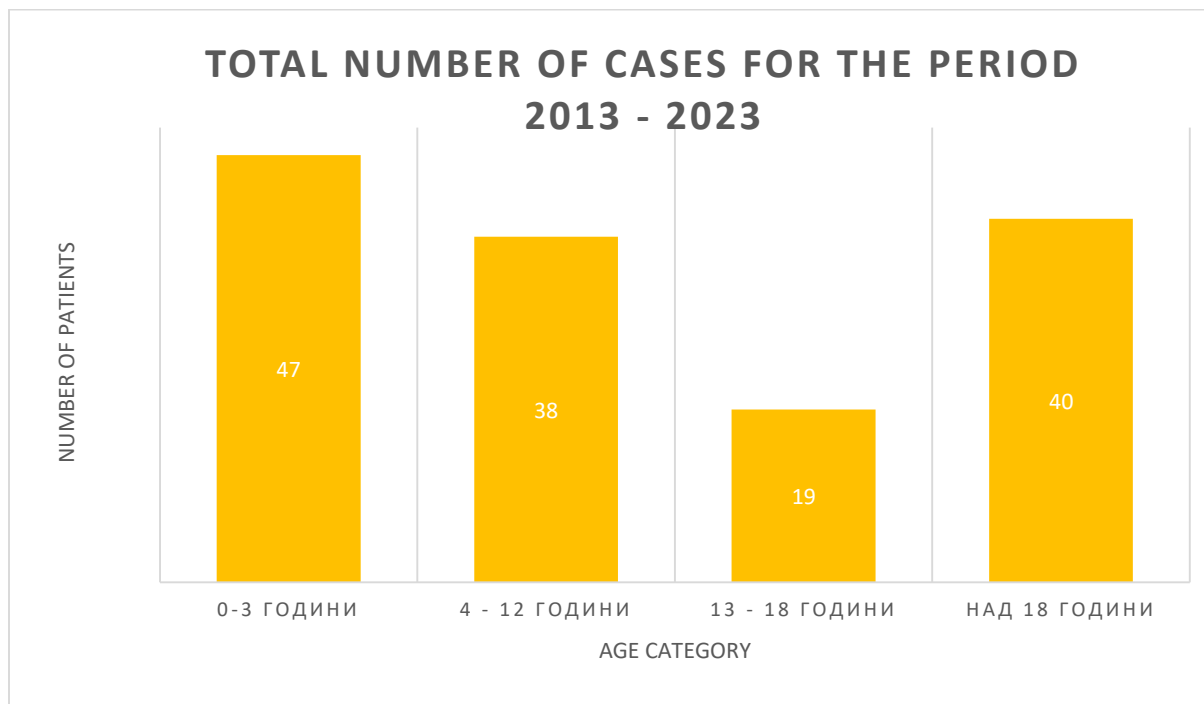
determine the level of antimicrobial resistance of clinical isolates of *C. jejuni*, the most frequent cause of campylobacteriosis in young children and elderly patients (65 years) in Bulgaria.

## Materials and methods

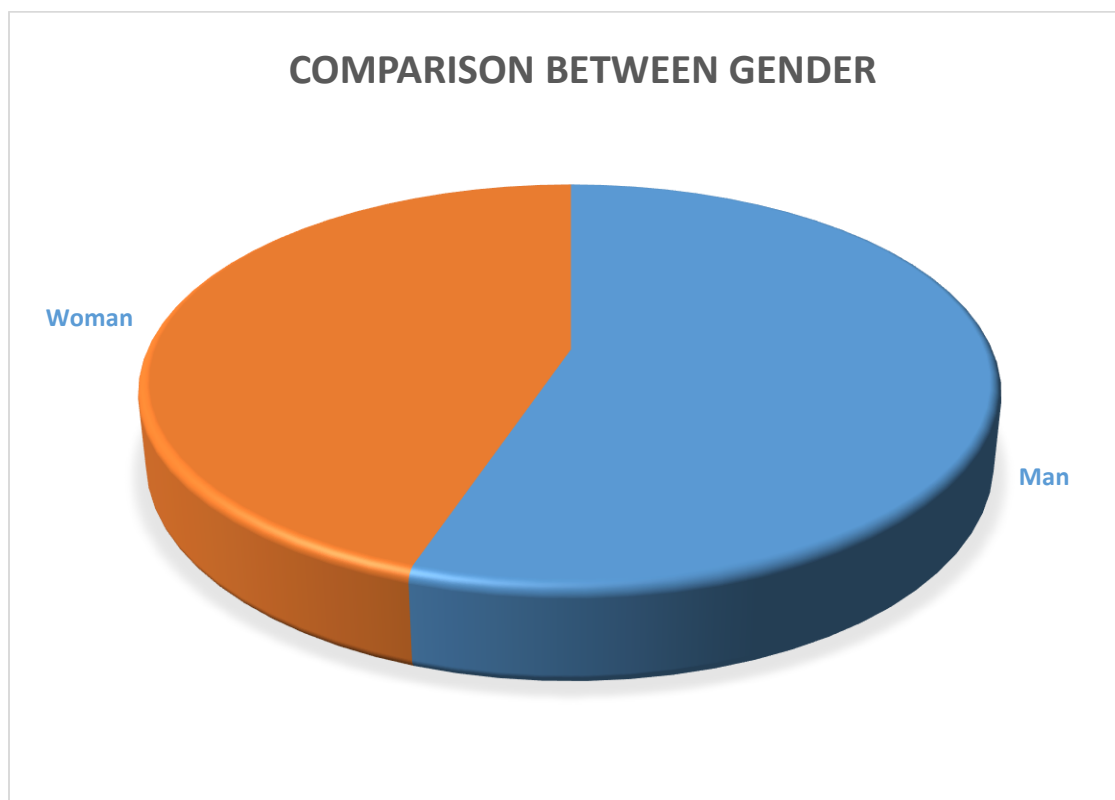
More than 350 *C. jejuni* strains are isolated from all over Bulgaria for a period of 10 years (2013-2023). The strains were isolated from patients with mild to severe diarrhoeal syndrome from 0 to 91 years of age. Microbiological studies on *Campylobacter jejuni* are carried out by plating on 5% sheep blood agar (Becton Dickinson GmbH) and cultivating at 42°C for 48-72 hours under microaerophilic conditions. Micro aerobic atmospheres of 5–10% oxygen, and 5–10% carbon dioxide are required for optimal growth (OIE, 2008). Appropriate atmospheric conditions can be generated by a variety of methods, and gas-generating packages were utilized in this study. Identification of *Campylobacter* cells was performed using the MALDI-TOF system and biochemical tests- a colony from the bloody plate was tested for catalase, oxidase, hippurate, and indoxyl acetate. To conduct antimicrobial susceptibility testing, antibiotic discs including Ciprofloxacin, Tetracycline, Nalidixic acid, Erythromycin, Cefuroxime, and Azithromycin were used. Each confirmed strain was tested for susceptibility to the antibiotics in question.

## Results

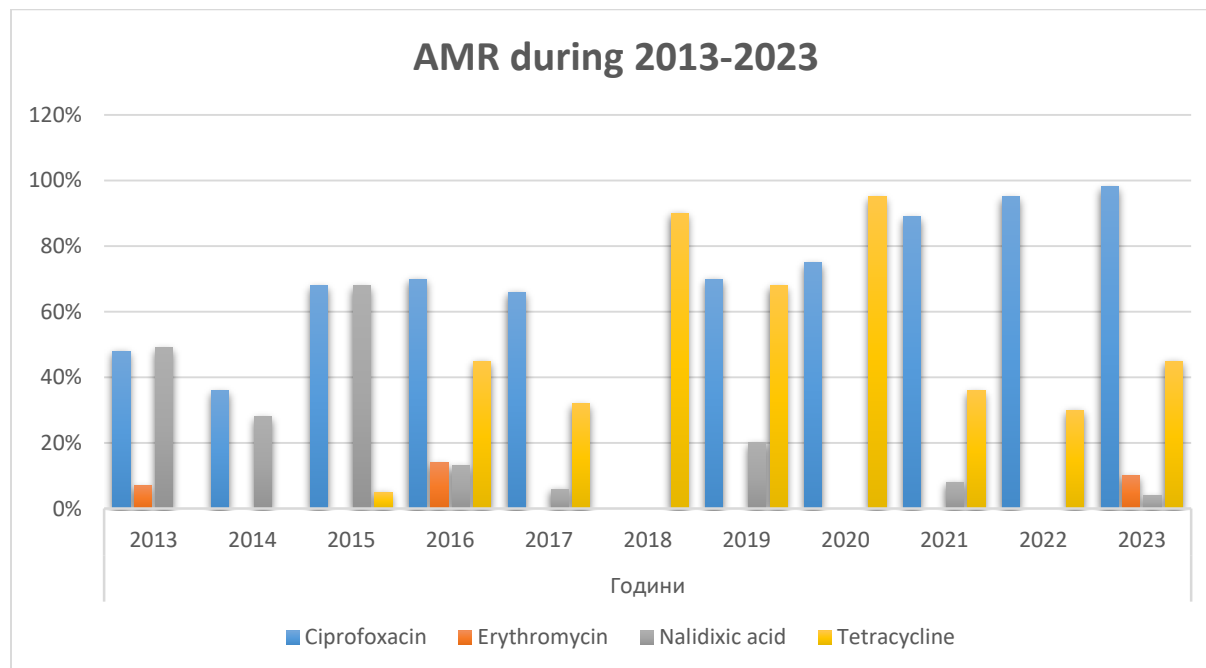
This study is focused on observing the emergence of antimicrobial resistance (AMR) in clinical isolates of *C. jejuni* from all regions of Bulgaria over a 10-year period. Among individuals of different ages and sexes, more than 350 strains of *C. jejuni* have been isolated (Graph 1, Graph 2). This bacteria was more frequently infective in males than females, with the highest incidence rate being observed in children aged 0 to 3. The analyses revealed high levels of resistance to Ciprofloxacin that exceeded 80% of the resistance level among other antibiotics, and they persist (Graph 3). A 5% increase in erythromycin resistance has been confirmed in Bulgarian human isolates of *C. jejuni*, according to the results of the study (Graph 3).



**Graphic 1.** Total number of cases for the period 2013 – 2023.



**Graphic 2.** Comparison between gender.



**Graphic 3.** Resistance over the last 10 years.

## Discussion

The international study confirms our findings that *C. jejuni* is the leading cause of campylobacteriosis among humans, and it requires intensive epidemiological surveillance. The subject of surveillance is extensive in the developed countries of the European Union, but this study is sparse in Bulgaria. The main cause of this is the lack of diagnosis and reporting of cases, as well as the absence of studies on this pathogen. Due to these circumstances, the main objective of this study is to investigate the frequency of isolated antibiotic resistance among *Campylobacter jejuni* species. Our findings on the prevalence of *Campylobacter jejuni* in patients with diarrheal syndrome are in accordance with those reported in the literature. The group of individuals who are between the ages of 0 and 3 years is the most vulnerable to these diseases. Campylobacteriosis has been a prevalent infectious disease in the European Union since 2005, compared to approximately 1.3 million cases per year in the United States. Consuming raw milk, undercooked chicken products, contaminated water, and contact with animals are all associated with *Campylobacter* infection [1]. Acute watery diarrhea, lasting from 2 to 7 days, is usually reported

by patients, and it may be accompanied by blood, mucus, and occasionally pus in the stool. High fever is also a common symptom of *Campylobacter* infection, with values of up to 40°C in children under 5 years of age [2]. Clinical manifestations of campylobacteriosis include diarrhea, Crohn's disease, Miller-Fisher syndrome, and neurological complications like reactive arthritis and Guillain-Barre syndrome. The immunocompromised and patients over 60 years old are at highest risk of morbidity, mortality and prolonged illness. The outcome of the disease depends on the immune status of the patient and the use of antibiotics is necessary to treat severe clinical cases in children, elderly and immunocompromised individuals [3,4]. Antibiotic resistance of *Campylobacter spp* can evolve by spontaneous mutations, natural transformation, transduction or conjugation, depending on the different mechanisms of evasion of each antibiotic [5]. For many years, fluoroquinolones were used as the first choice in the therapy of campylobacteriosis. This group of antibiotics has a broad spectrum of action and has efficacy against both gram-negative and gram-positive bacteria [6]. In livestock, fluoroquinolones are commonly used as a feed additive and also as a means of treating infections [7,8]. As a result of their overuse, multidrug-resistant (MDR) pathogens are increasingly emerging worldwide [9]. On the other hand, a single point mutation in the quinolone resistance determining region (QRDR) of the *gyrA* gene is thought to be a mechanism for the development of ciprofloxacin resistance [10,11]. In our study, we confirm the international data on the rapidly increasing antimycobacterial resistance to fluoroquinolones with more than 80%, particularly in ciprofloxacin. That is why The World Health Organization (WHO) includes *Campylobacter spp* in the list of bacteria for which new antibiotics are urgently needed and classifies them as high-priority pathogens due to the emergence worldwide of strains with a high level of resistance to fluoroquinolones [12]. This increase in fluoroquinolone resistance necessitates the introduction of a new class of antibiotics. Nowadays, macrolides are the primary method of treating campylobacteriosis. [13]. Erythromycin has been introduced as a substitute in the clinical management of human campylobacteriosis due to increased resistance to ciprofloxacin [14]. On the other hand, erythromycin resistance rates have been increasing in recent years [15,16], which calls for urgent active surveillance for antibiotic resistance. In our study erythromycin shows only 5 % AMR for last 10 years. The Covid-19 pandemic in Bulgaria may have been a result of the excessive use and misuse of antimicrobials. Macrolide resistance is a major issue in the treatment of human infections, as erythromycin is the primary antibiotic used for human treatment. [17].

## Conclusion

In conclusion, we recommend regular monitoring of *Campylobacter jejuni* resistance and strict implementation of etiologic antimicrobial therapy.

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