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Antibiotic Resistance in Clinical Isolates of Campylobacter

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Abstract

Campylobacter spp. are the most commonly isolated bacterial agents of acute diarrhea in the world.

Materials and methods: For 2017, 260 patients were hospitalized with diarrhea syndrome. Of these, 66 (25.38%) were positive for *Campylobacter* spp. by cultured and Multiplex PCR. Clinical isolates were tested for susceptibility against 5 antimicrobials.

Results: The isolates of *Campylobacter* 60 (91%) are *C. jejuni* and 6 (9%) – *C. coli*. All *C. jejuni /coli* isolates are Azithromycin (AZI) sensitive, and all *C. coli* isolates are susceptible to Clarythromycin (CLA) and Erythromycin (ER). Three isolates of *C. jejuni* (5%) are resistant to CLA and 7 (11.6%) – to ER. To Ciprofloxacin (CIP) are resistant 35 (58.3%) of *C. jejuni* isolates and 2 (33.3%) of *C. coli*. For Tetracycline (TE) the resistance is 23 (38.3%) for *C. jejuni* and 1 (16.6%) for *C. coli*, respectively. In *C. jejuni* there were 3 multiresistant isolates (5%) to CIP-CL-ER-TE simultaneously; also 3 (5%) to CL-ER-CIP and 7 (11.6%) to TE-ER. In *C. coli* there was 1 (16.6%) TE-ER-resistant isolate.

Discussion: Increased resistance of *Campylobacter* spp. to the most commonly used antimicrobial agents has been observed. Particularly worrying is the high resistance to Ciprofloxacin, as well as the increasingly common multi-drug resistant isolates.

Keywords: *Campylobacter*, Ciprofloxacin, Antibiotics, Tetracycline.

Introduction

Infection with *Campylobacter* spp. is the leading cause of bacterial gastroenteritis in Europe and North America. It is a typical food infection with a source of domestic and wild birds, mainly broilers, the human disease most often caused by the so-called crossbreeding in the preparation of various foods being in contact with poultry meat. Less often the source may be unpasteurized milk, contaminated drinking water, pets or a sick person. Campylobacteriosis is also one of the causes of diarrhea in travellers [1-5].

Leading agents of diarrheal diseases in humans are *Campylobacter jejuni* and *Campylobacter coli*. Although rarely *C. jejuni* is described as a cause of some autoimmune diseases such as Guillain-Barre syndrome, post-infectious arthritis, neuropathies, etc [1]. In practice, macrolides (Azithromycin, Clarithromycin), especially fluoroquinolones (Ciprofloxacin) are most commonly used in antimicrobial therapy

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of children and adults. A number of authors describe tetracyclines as a possible choice, but in practice they are seldom applied. Due to the frequent use of fluoroquinolones in broiler breeding and the abuse of antibacterial therapy, numerous studies have shown a sharp increase in resistance to fluoroquinolones, tetracyclines and even some macrolides [1-5]. The purpose of this study is to detect the prevalence and antimicrobial resistance of *C. jejuni* and *C. coli* isolated from hospitalized patients with diarrheal syndrome.

Materials and Methods

Collection of samples and reference strains

The bacterial strains used as positive controls in this work are ATCC 33560 for *C. jejuni* and C-14.2 for *C. coli*.

In 2017, 260 faecal samples were collected from patients hospitalized with diarrheal syndrome aged 0-80.

Rapid test and sample processing

Each faeces was tested by an immunochromatographic assay of CerTestBiotec, S.L. according to the manufacturer's instructions. Of all positive samples, plain blood agar with 10% defibrillating sheep blood (BulBio, Bulgaria) was performed on a membrane with nitrocellulose membranes (SartoriusStedimBiotech) with a pore size of 0.45 μm. The seeds were cultured in a microaerophilic atmosphere (5% O_2 + 10% CO_2 + 85% N_2), which was generated from gas – again "Helyco-Campy Pac" in anaerobic jar (Oxoid, USA) at 42-43°C for 24-72 hours.

Phenotypic identification of *Campylobacter* spp.

For the validation tests according to *ISO 10272-1: 2006* and *ISO/TS 10272-2: 2006* pure culture was used. We used a catalase and oxidase test, hydrolysis of sodium hippurate and indoxil acetate.

DNA extraction and PCR identification

For isolation of DNA from fresh culture, lysis buffer PrepMan™ Ultra (Applied Biosystems, Foster City, CA) was used. Three pairs of primers, identifying and differentiating strains of *C. Jejuni/C. coli* were used for Multiplex – PCR analysis. The primers amplifying the genes have the following sequences: for cadF-F-TTGAAGGTAATTTAGATATG, for ***cadF-R***-CTAATACCTAAAGTTGAAAC; for ***hipO***-F-GAAGAGGGTTTGGGTGGTG, for ***hipO***-R-AGCTAGCTTCGCARAATAACTTG; for ***asp-F***-GGTATGATTTCTACAAAGCGAG, for ***asp-R***-ATAAAAGACTATCGTCGCGTG. The final reaction volume was 25 μl and in this volume were included 1xTaqDNAPolymerase buffer; 4mM MgCl2; 0.2 mM dNTP; 0.03 U/μl of SuperHotTaq DNA Polymerase (*set Applichem GmbH, Germany*); 0.6 μMcadF-F/R; 0.2μMasp-F/R; 0.2 μMhipO-F/R and 5 μl DNA. The temperature regime was 94°C for 5 min. – initial denaturation, followed by 35 cycles: 94°C – 45 sec. – denaturation, 52°C – 45 sec. – hybridization, 72°C – 60 sec. – elongation, 72ºC – 2 min – final elongation step. All PCR products in the assay methods were split into standard agarose electrophoresis in 1 x TBE buffer at 6V/cm, 100 Ams in 2% agarose gels. Visualization of the amplified products and molecular markers was performed by illuminating by ultraviolet a light transilluminator.

Antibiotic sensitivity test

We used the Kirby-Bauer standard disk-diffusion method as the test was performed according to the recommendations and standards of the CLSA Guidelines. For the interpretation of the size of the inhibition zones, we used the CLSA standards related to the *Enterobacteriaceae* family.

Results

Of the 260 probes tested, 66 (25.38%) were positive for *Campylobacter* spp., of which 60 (91%) were identified as *C. jejuni* and 6 (9%) as *C. coli*.

Isolates were tested for susceptibility to 5 antimicrobials used in clinical practice to treat *Campylobacter* spp. infection. The data interpretation revealed that all isolates are susceptible to at least one of the antimicrobial agents. All *C. jejuni/coli* isolates are Azitromycin (AZI) sensitive, and all *C. coli* isolates are susceptible to Clarithromycin (CLA) and Erythromycin (ER). Three isolates (5%) of *C. jejuni* are resistant to CLA and 7 (11.6%) to ER. 35 (58.3%) of C. jejuni isolates are resistant to Ciprofloxacin (CIP) and 2 (33.3%) of *C. coli*. For Tetracycline (TE), the resistance is 23 (38.3%) for *C. jejuni* and 1 (16.6%) for *C. coli*. In *C. jejuni* there were 3 (5%) isolates multi-resistant to CIP-CL-ER-TE simultaneously; also 3 (5%) – to CL-ER-CIP, and 7 (11.6%) – to TEE-ER. In *C. coli* there was 1 (16.6%) TE-ER resistant isolate (Table 1).

Discussion

Although campylobacteriosis often manifests itself as a mild self-limiting diarrheal disease, it is common for children and immunocompromised patients to develop as severe gastroenteritis or severe extra-intestinal complications. In the case of severe circuit and underlying diseases, the antimicrobial treatment of the infection is mandatory, with macrolides and fluoroquinolones being used most frequently in clinical practice [1]. In Bulgaria, the very commonly used agents are Clarithromycin, Erythromycin and Ciprofloxacin [6]. The antimicrobial resistance of some *Campylobacter* strains is becoming increasingly common in the world. *Campylobacter jejuni* has a relatively low resistance to ER – about 5% and is one of the most widely used drugs in the European Union (EU). The worrying data on the increase in CIP-resistant strains have been known since the end of the 20th century. Currently, in the EU, it has reached an average of 47.4%. Due to the fact that resistance to fluoroquinolones is due to two synergistic mechanisms, fluoroquinolone resistant mutants develop rapidly during antibiotic treatment, and the strains persist even after removal of the selection [1,5].

Table 1: The frequency of antibiotic drug resistance in *Campylobacter* spp.

Resistance profile	Resistant isolates			
	C. jejuni		*C. coli*	
	No.	(%)	No.	(%)
Ciprofloxacin	35/60	(58.33)	2/6	(33.33)
Azithromycin	0/60		0/6	
Clarithromycin	3/60	(5)	0/6	
Erythromycin	7/60	(11.66)	0/6	
Tetracycline	23/60	(38.33)	1/6	(16.66)

As reported by other EU countries, our data also show an alarmingly high resistance of *C. jejuni* strains to CIP. Although significantly lower, *C. jejuni*'s resistance to ER also occurs in our country approximately as in other European countries. According to many authors, the resistance of *C. coli* to macrolides is higher than that of *C. jejuni*, but in our study we do not find such regularity. It is very possible that this is due to the small number of *C. coli* strains we have worked with. None of the strains of *Campylobacter* spp. in our work did show resistance to AZI, which is a prerequisite for it to be most commonly seen as a means of choice when using macrolides. Tetracyclines are rarely the first choice in the treatment of campylobacteriosis, and the high resistance of our strains to it is comparable to that in other EU countries [3].

Very soon, in 2017, the World Health Organization announced the list of the world's most problematic bacteria for which it is urgently needed to develop new antibiotic molecules, such as fluoroquinolone-resistant *Campylobacter* spp. which are ranked fourth in the "high priority" category [5]. In recent years, multi-resistant strains of *Campylobacter* spp. have also been reported more frequently, and our isolates have also been reported in our work.

Conflict of Interest: None

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