

## PRESENCE OF RESISTANCE IN CAMPYLOBACTER JEJUNI AND CAMPYLOBACTER COLI

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There are 18 species belonging to the genus of *Campylobacter* (rRNK group I), of which thermophilic ones are the following: *Campylobacter jejuni*, *Campylobacter coli*, *Campylobacter lari* and *Campylobacter upsaliensis*. The aim of our research was to determine the sensitivity of *Campylobacter* species, isolated from human feces, to antibiotics being used in practice. The study involved 50 human strains of *C. jejuni/coli* isolated from feces in the Centre for Microbiology at the Public Health Institute Nis. Sensitivity was tested by applying the disk diffusion method on seven antibiotics (erythromycin, gentamicin, tetracycline, ciprofloxacin, chloramphenicol, cephalixin and nalidixic acid). Our results showed low resistance to erythromycin, gentamicin and tetracycline (2%), which corresponds to the studies conducted in the world. Moreover, these findings indicate that erythromycin may be considered as the drug of choice in the treatment of *Campylobacter diarrhea* in this region. Resistance to fluoroquinolone and nalidixic acid was 44%, and *C. coli* showed higher resistance compared to *C. jejuni*, though statistical significance was not proved. *Acta Medica Medianae* 2009;48(2):14-17.

**Key words:** *Campylobacter jejuni*, *Campylobacter coli*, resistance, disk diffusion, antibiotics

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### Introduction

There are 18 species belonging to the genus *Campylobacter* (rRNK group I), and among them the thermophilic ones are: *Campylobacter jejuni*, *Campylobacter coli*, *Campylobacter lari* and *Campylobacter upsaliensis* (1). Those bacteria grow best on 42 °C (2).

*Campylobacter jejuni* (*C. jejuni*) and *Campylobacter coli* (*C. coli*) are important causes of diarrheal illness in all parts of the world (3). They are prominent cause of traveler's diarrhea (3). In the United States, these infections affect approximately 1% of the population each year (4), with an estimated rate of 2.4 million cases per year (5). Studies conducted in England have shown that more than 90% of infections in England are caused by *C. jejuni*, followed by infectious caused by *C. coli*. *C. lari* comprises less than 1% of the strains isolated (6).

Campylobacteriosis is primary zoonotic disease, with raw and undercooked poultry being an important source of campylobacter infection. Contaminated milk, water, pork, beef, lamb and seafood also contribute to human infections. *C.*

*coli* is normal inhabitant of pig's gastrointestinal system (7); *C. jejuni* is isolated in birds (2), while *C. upsaliensis* is associated with dogs (6).

However, the frequency and pattern of occurrence are different between developed and developing countries (especially in the number of cases reported in adults and the presence of any seasonal patterns in occurrence). In developing countries, most infections occur in children, while in developed countries campylobacteriosis predominate in adults. Previously reported prevalence rate of *Campylobacter spp.* in children in Southeast Asia was in the range from 2.9-15% (8).

Symptoms of campylobacteriosis usually seen in humans are diarrhea, fever, abdominal pains, cell exudates and blood in stool. However, illness can manifest as septicemia or myocarditis, pancreatitis, meningitis, cholecystitis, arthritis and erythema nodosum. The clinical complications of campylobacter infections include toxic megacolon, hemolytic uremic syndrome, Reiter's syndrome and Gullian-Barré syndrome (9). Most cases of campylobacter enteritis do not require antimicrobial treatment. It is usually short in duration, clinically mild and self-limited. However, some cases of these infections require treatment: severe and prolonged enteritis, septicemia and other extra intestinal infections (4). Also, antimicrobial treatment is necessary for campylobacter infections in immunosuppressed patients (10).

When the therapy is necessary, drug of a choice is erythromycin. Tetracycline, fluoroquinolone, chloramphenicol, ampicillin and gentamicin are often used as alternative drugs for treatment (4).

Within last few years, increased level of *Campylobacter* resistance to antibiotics, especially to erythromycin, fluoroquinolones and beta-lactamase, has been noticed. Occurrence of resistance is associated with use of macrolides and quinolones in veterinary medicine (5). In the 1980, fluoroquinolones were introduced in therapy of enteritis caused by *campylobacter*, and were effective against most major pathogens causing bacterial enteritis. Fluoroquinolones initially showed good *in vitro* activity against thermophilic *Campylobacter* species. However, resistance of *Campylobacter* species toward these antibiotics increased, sometimes after only one or two treatments, whereas diarrhea caused by the resistant strains has longer duration (4).

### Aims

The aim of our study was to determine the resistance of *C. jejuni* and *C. coli* to erythromycin, gentamicin, tetracycline, ciprofloxacin and chloramphenicol, used in therapy as well as cephalixin and nalidixic acid, used for identification.

### Material and methods

Research included 50 strains isolated and identified from human feces in the period 2003-2007 at the Public Health Institute Nis. Isolation of thermophilic species of *Campylobacter* was conducted by culturing specimens on Skirrow agar (bioMérieux, France) and incubated at 42°C in microaerophilic atmosphere for 48 hours. For identification we used colony morphology (small, smooth, radiant, flat or convex colonies, confluent on the fresh media when they have a water drop look), microscopic examination (Gram-negative, curved bacilli, S-shaped), biochemical tests (oxidase test, catalase test, hippurate hydrolysis and automatic identification system API-Campy, bioMérieux, France).

Sensitivity of the strains to antibiotics was examined by disk-diffusion method on blood agar enriched with 5% of sheep blood. Suspension was prepared by inoculation of full loop into 5ml of a sterile saline and adjustment of density to 5 McFarland standard. Disks were applied by forceps; distance between centers of two adjacent disks were 3 cm, and a distance from the edge of the plate was 1cm. Plates were incubated at 37°C in microaerophilic conditions achieved by GasPak-system (bioMérieux, France) within 48 hours. Having in mind that the size of inhibition zone and break-points for *campylobacters* are not yet standardized, we used recommendations for *Staphylococcus aureus* standard for erythromycin, and standard for *Enterobacteriaceae* for the rest of antibiotics according to Clinical Laboratory Standards Institute USA (CLSI), 2008. (11). We used disks of tetracycline (30 µg) (Bioanalyse, Turkey), erythromycin (15 µg), gentamicin (10 µg), nalidixic acid (30 µg), ciprofloxacin (5 µg), chloramphenicol (30 µg) and cephalixin (30 µg) (Neo sensitabs<sup>tm</sup>, Rosco-Diagnostica, Danmark).

Statistical data were processed by Epi info software, version 5.

### Results

Out of 50 strains, 35 *C. jejuni* strains and 15 strains of *C. coli* were examined. Twenty-seven isolated strains were resistant to one antibiotic, two strains were resistant to two, and 19 isolates were resistant to three antibiotics.

In 35 isolates of *C. jejuni*, 19 were resistant to one antibiotic (54.3%), two were resistant to two antibiotics (5,7%) and 12 were resistant to three antibiotics (34.3%). Of 15 isolates of *C. coli*, eight were resistant to one antibiotic (53.3%), and seven were resistant to three antibiotics (46.7%).

Table 1. Sensitivity of thermophilic *Campylobacter* spp. to examined antibiotics

Antibiotics	Sensitivity of strains			
	S		R	
	Number of isolates	%	Number of isolates	%
erythromycin	49	98	1	2
gentamicin	49	98	1	2
tetracycline	49	98	1	2
ciprofloxacin	28	56	22	44
nalidixic acid	28	56	22	44
chloramphenicol	48	96	2	4
cephalexin	7	14	43	86

S-Sensitive

R-Resistant

Table 2. Sensitivity of *C. jejuni/coli* to examined antibiotics

Antibiotics	Sensitivity of strains			
	<i>C. jejuni</i>		<i>C. coli</i>	
	%		%	
	S	R	S	R
erythromycin	97,1	2,9	100	0
gentamicin	97,1	2,9	100	0
tetracycline	97,1	2,9	100	0
ciprofloxacin	57,2	42,8	53,3	46,7
nalidixic acid	57,2	42,8	53,3	46,7
chloramphenicol	94,4	5,6	100	0
cephalexin	20	80	0	100

S-Sensitive

R-Resistant

Resistance to erythromycin, gentamicin and tetracycline was represented in low percentage (Table 1). Higher levels of resistance to nalidixic acid and ciprofloxacin were seen in *C. jejuni* (42.8%), and in *C. coli* (46.7%) (Table 2). There were not statistically significant differences between sensitivity to nalidixic acid and to ciprofloxacin within *C. jejuni* and *C. coli* strains ( $p=0.8773907$ ;  $\chi^2=0.02$ ).

In patients up to 7 years of age (27 pts), resistance to ciprofloxacin was 44.4%. In patients from 7 to 15 years of age (7 pts), resistance to ciprofloxacin was 28.6%. In adult patients (16 pts), resistance to ciprofloxacin was 50%. Chi-square test showed that there was no statistically significant difference between investigated age groups in resistance to ciprofloxacin.

## Discussion

Our strains showed low resistance to erythromycin, gentamicin and tetracycline (2%). Resistance data of *C. jejuni* and *C. coli* to erythromycin are different in different parts of a world. Majority of studies emphasize higher resistance of *C. coli* to erythromycin (up to 68.4%) compared to *C. jejuni* (up to 11%). However, we did not find this in our research. Low and stable resistance of thermophilic campylobacters to erythromycin has been found in Japan, Canada and Finland, while resistance is increasing in Sweden and Taiwan (2). Studies conducted in the Arab Gulf, showed a high level of sensitivity of isolated strains to erythromycin, confirming erythromycin as a drug of choice for enteritis caused by campylobacters (12). However, in Northern Ireland, mild increase of campylobacter resistance to erythromycin (4.2%) in human population was noted (13).

According to some studies, it seems that resistance to gentamicin is low. In a study conducted by Ge Beilei et al., which included 81 isolates of *C. jejuni* and 54 isolates of *C. coli* obtained from raw retail meat, resistance to gentamicin was not noticed (5). In the study conducted in Spain in the period from 1997 to 1998, resistance of human strains to gentamicin was 0.4% for *C. jejuni* and 8.6% for *C. coli* (14). Similar studies on pediatric patients, in Spain, too, revealed resistance to gentamicin in 1% of isolated strains (15).

Data obtained from Sweden showed that in the period 2000-2002 the resistance to tetracycline was 4%. In other European States it was up to 39%, and in some Asian States up to 87% (16). Highest level of resistance was recorded in the USA for *C. jejuni* (85.2%), and for *C. coli* (77.8%) (5).

Our strains showed resistance to ciprofloxacin in 44%. Resistance to fluoroquinolones has increased from 1989 by introducing enrofloxacin in veterinary as well as broad application of fluoroquinolones in human medicine in Europe (Holland, France, Spain). In regions where the resistance to fluoroquinolones is endemic, these antibiotics are not recommended in the therapy of diarrhea (2). In 1993, in Northern Ireland, resistance to fluoroquinolones was 3.8%. Moreover, resistance increased up to 10% until 1995 (13).

Our strains were resistant to nalidixic acid in 44%. In Northern Ireland, from 1980 to 1991, resistance to nalidixic acid was not detected (13). In the USA, detected resistance to nalidixic acid was 1.3% in 1992 and 10.2% in 1998 (8). However, one study conducted in Northern Spain in 2005 revealed resistance up to 76% (17).

Our strains are sensitive to chloramphenicol in high percentage (98%). Many studies have shown high activity of chloramphenicol *in vitro* against most strains of campylobacters. Sagara and et al. emphasized important differences in sensitivity among *C. jejuni* and *C. coli* strains. Namely, there was no resistance in *C. jejuni* strains, while in *C. coli* it was recorded in 10% (15). In Northern Ireland, resistance to chloramphenicol was noticed in 1993 for the first time in 1.4% examined strains. In Kenya, 24% strains of campylobacters showed resistance to this drug (8).

Our *C. jejuni* strains were resistant to cephalexin in 80%, while *C. coli* strains were 100% resistant. Pezzotti et al. confirmed that 100% of human strains were resistant to first generation of cephalosporins (18).

## Conclusion

Since most of our strains were sensitive to erythromycin, gentamicin, tetracycline, and ciprofloxacin, besides erythromycin as a drug of choice, these antibiotics can be used in the therapy of diarrhoea caused by thermophilic campylobacters. However, resistance to quinolones diminishes their application and urges necessities of antibiotic susceptibility testing, though resistance to nalidixic acid limits its use in identification of our strains.

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## POJAVA REZISTENCIJE KOD BAKTERIJA *CAMPYLOBACTER JEJUNI* I *CAMPYLOBACTER COLI*

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Od 18 vrsta koje pripadaju rodu *Campylobacter* (rRNK grupa I), termofilne su: *Campylobacter jejuni*, *Campylobacter coli*, *Campylobacter lari* i *Campylobacter upsaliensis*. Cilj našeg ispitivanja bio je utvrđivanje osetljivosti termofilnih vrsta kampilobaktera izolovanih iz humanog fecesa na antibiotike koji se koriste u praksi. Ispitivanjem je obuhvaćeno 50 humanih izolata *C. jejuni/coli* izolovanih u Centru za mikrobiologiju Instituta za javno zdravlje u Nišu. Osetljivost je testirana primenom disk difuzione metode na 7 antibiotika (eritromicin, gentamicin, tetraciklin, ciprofloksacin, hloramfenikol, cefaleksin i nalidiksična kiselina). Rezultati su pokazali nisku rezistentnost na eritromicin, gentamicin i tetraciklin (2%), što odgovara studijama sprovedenim u svetu i govori u prilog tome da se eritromicin i dalje može smatrati lekom izbora u lečenju dijareja izazvanih kampilobakterom na našim prostorima. Rezistentnost na fluorokvinolone i nalidiksičnu kiselinu iznosila je 44% pri čemu su veću rezistentnost pokazali izolati *C. coli*, u odnosu na *C. jejuni*, ali statistički značajna razlika nije dokazana. *Acta Medica Medianae* 2009;48(2):14-17.

**Ključne reči:** *Campylobacter jejuni*, *Campylobacter coli*, rezistencija, disk difuzija, antibiotici