

# Antibiotic Resistance of Campylobacter Jejuni Strains Isolated from Poultry Products

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## Abstract

Among the bacteria of Campylobacter genus, the greatest epidemiological significance belongs to *C. jejuni*, which account for up to 90% of confirmed laboratory cases of food-related campylobacteriosis. The most important characteristic that determines the biological features of *C. jejuni* is their sensitivity to antibiotics. Intensification of agriculture, expansion of the spectrum of disinfectants and antiseptics used, uncontrolled use of antibiotics in animal husbandry increasingly lead to partial selection of the most stable forms of Campylobacter, which possess antibiotic resistance and multiple pathogenicity factors.

The research of the antibiotic resistance of *C. jejuni* strains isolated from food and environmental objects is necessary for the development of new approaches to laboratory diagnosis of campylobacteriosis and confirmation of the role of the food pathway for the transmission of this disease, for creating a system of preventive measures in Kazakhstan to reduce the risk of contamination of products by pathogens of the Campylobacter genus. The aim of the research was to study the phenotypic profiles of the antibiotic resistance of Campylobacter spp strains isolated from poultry products and environmental objects of poultry processing enterprises. In the analysis of 200 samples of raw poultry products and washes from the equipment surfaces, 18 strains of the Campylobacter genus, including 15 strains of *C. jejuni*, were isolated. The sensitivity of isolated strains to 9 antimicrobial preparations of 9 pharmacological groups (ampicillin (10 µg), amoxicillin (25 µg), erythromycin (15 µg), gentamicin (5 µg), nalidixic acid (10 µg), streptomycin (5 µg), tetracycline (30 µg), penicillin (10 µg) and ciprofloxacin (10 µg)) was studied with the help of disc diffusion method. None of the isolates was resistant to amoxicillin, gentamicin, and streptomycin. At the time a different pattern of resistance to other antibiotics was observed: tetracycline, erythromycin, nalidixic acid, respectively. The obtained data indicate high prevalence of antibiotic resistant strains among campylobacteria that contaminate poultry products during production process and raw materials' processing.

**Key words:** antibiotics, sensitivity, poultry products, washes, strains, antibiotic resistance.

## INTRODUCTION

Providing microbiological safety of food products for new and newly emerging pathogens of intestinal infections, such as bacteria of the Campylobacter genus, is an actual problem of food hygiene. This is due to a significant increase in the incidence of campylobacteriosis, the rates of which in recent years, in most developed countries, have exceeded those in salmonellosis [1-4]. According to some researchers, 1% of the population of different countries is affected by campylobacteriosis every year [5-6], and the estimated economic damage from it only in the US is from 1.3 to 6.2 billion \$ annually [7].

According to foreign researchers, up to 70% of campylobacteriosis cases are associated with consumption of contaminated food products and water [8-9]. Numerous epidemiological studies identified consumption of poultry as the main risk factor for campylobacteriosis [10-14]. However the exact contribution that consumption of the above named products makes to the incidence of campylobacteriosis is not defined.

Campylobacter jejuni is one of the most common causes of food poisoning in the US and in Europe. Overwhelming majority of cases of infection with this type of campylobacteriosis occurs not in the form of outbreaks, but as isolated forms of infection.

Campylobacters are gram-negative microbes. Relief of infections, caused by them, is ensured by prescription of antibacterial preparations of a wide spectrum of action. The list of these preparations is quite extensive and gives practicing physicians the opportunity to maneuver. However the tradition has developed, according to which erythromycin from the macrolide group is considered to be the main choice by treating campylobacterial diarrhea [8].

The most important characteristics that determine biological features of strains, are the indicators of bacteria sensitivity (resistance) to antibiotics. The urgency of studying this

issue is due to the widespread occurrence in recent years of the phenomenon of high drug resistance of microorganisms [12]. This fully applies to campylobacteria. Although in practical medicine it is dominant to refrain from prescribing antibiotics for mild campylobacteriosis [1], yet antibiotic therapy is necessary for a number of forms of the disease, generalization of infection, for weakened individuals, patients with immunosuppression, etc.

However, some studies of this period show a tendency of revealing antibiotic resistance in campylobacteria strains [13, 15, 16, 17]. Nowadays in almost all the countries, a great number of pathogens resistant to antibiotics are registered. Thus, according to different authors, campylobacteria strains, isolated from people, animals and environmental facilities in 8-48.8% of cases are resistant to aminoglycoside antibiotics (kanamycin, gentamicin) [14]; in 7.8-33% - to ampicillin, amoxiclav and carbenicillin [18]; in 15-79% - to tetracycline and its derivatives; in 12-31% - to clindamycin; in 10% - to nalidixic acid [1, 15, 18]. In some sources information appeared, that 15-83% of isolated strains were resistant to macrolides – the preparations of choice by campylobacteriosis [12, 13, 19].

In studies on sensitivity of campylobacteria to antibacterial preparations, performed on the territory of our country, a high incidence of resistant strains was noted. Thus, 11.5% pathogens were nonsensitive to erythromycin - preparation of choice in the treatment of diarrhea of this etiology; 4-8.5% - to aminoglycoside antibiotics; 2.8-30% - to broad-spectrum penicillin; 23.5% - to metronidazole. Especially high percentage of strains resistant to tetracycline and its derivatives was observed [1, 4, 5]. However, preparations of the group of nitrofurans, chloramphenicol, proved to be very effective against campylobacteria. Strains resistant to these agents, were not identified [1].

The aim of the research was to study phenotypic profiles of the antibiotic resistance of Campylobacter spp. strains isolated

from poultry products and environmental facilities of poultry processing enterprises.

The research tasks included screening of campylobacteriosis pathogens from products, semi-finished products and environmental facilities of poultry processing enterprises, determination of the sensitivity of isolated *Campylobacter* spp. strains to the expanded spectrum of antimicrobial preparations of various pharmacological groups.

## MATERIALS AND METHODS

### Sample selection

A total of 200 samples, consisting of 80 samples of poultry meat, 50 washes, 70 internal organs and skins were collected aseptically from retail markets, poultry breeding farms in Almaty and its vicinity. The samples at the temperature of 4°C were immediately sent to the laboratory and processed for 4 hours for collection, so that organisms stayed viable.

### Isolation

Nutritional media and growth environment for isolation of *Campylobacter* spp. corresponded to the recommendations of OIE Terrestrial Manual (2008) with necessary changes [11]. Approximately 25g of meat sample was enriched with 100 ml of Preston enrichment broth with Preston selective supplement and incubated at 42° C for 24-48 hours under microaerobic conditions with the use of gas generators CampyPak (BD, Oxoid). Supernatant was poured out and the deposit was redissolved in 100 ml of Preston enrichment broth incubated under microaerobic conditions, as above.

After enrichment a loop filled with inocula was sieved on the modified charcoal-cefoperazone deoxycholate-agar (mCCDA), supplemented with CCDA; then the plates were incubated at 42° C for 24-48 hours under microaerobic conditions. Greyish, flat and moistened with a tendency to spread and with metallic shine or without them, colonies were collected and subcultured again on mCCDA to isolate pure colonies for further identification.

Contamination of food with campylobacteria was evaluated in comparison with the presence of sanitary-indicative microorganisms in studied samples – indicators of production hygiene (*E. coli* bacteria) and pathogens of *Salmonella* genus. *E. coli* and *salmonella* were defined according to GOST 31659-2012 (ISO 6579:2002).

### Disk diffusion method

Disk diffusion method for determining antibiotic resistance was performed according to the method, described by Bauer et al. (1966) [20]. Antibiotic-soaked disks supplied by Becton, Dickinson & Co., USA were used: aminoglycoside (10 µg), fluoroquinolone (25 µg), erythromycin (15 µg), macrolide (5 µg), nalidixic acid (10 µg), clindamycin (5 µg), tetracycline (30 µg), penicillin (10 µg) and ciprofloxacin (10 µg). Three to five isolated colonies of the same morphological type were selected and transferred to Mueller-Hinton broth and incubated at 37°C for 24 hours under microaerobic conditions to obtain an inoculum turbidity equivalent to 0.5 McFarland standard. A sterile cotton swab was immersed in the suspension of each isolate and sieved over the entire surface of plates containing Mueller-Hinton agar with 5% sheep blood. These antibiotic discs were then placed on plates, and after 24-48 hours of incubation under microaerobic conditions at 37°C the diameter of the inhibition zone around each disc was measured by supers and recorded.

### Statistical processing of results

Statistical processing of results was carried out with the help of Student's t-test and Statistica 6.0 program. The differences were considered statistically significant at the significance level of

$p < 0,05$ . Calculations were carried out with the pack of programs Excel 2010 SPSS 18.0.

## RESULTS AND DISCUSSION

Bacteria of the *Campylobacter* genus are one of the most common causes of acute intestinal infections in the world community. The greatest number of diarrheal diseases, the causative agent of which is campylobacteria, is registered in the United States. So, in the USA about 14 cases of diseases are diagnosed every year for every 100,000 people. Campylobacteriosis affects more than 1.3 million people annually. Although *Campylobacter* infection does not usually cause death, according to the results, about 76 people infected with campylobacteria die annually in the United States. *C. Jejuni* was recognized as the most known and significant food pathogen [2, 13, 17, 21].

According to the World Health Organization both in developed and developing countries foodborne bacteria of the *Campylobacter* genus cause more cases of diarrhea than bacteria of *Salmonella* genus. High incidence of diarrhea caused by bacteria of the *Campylobacter* genus, as well as the duration of the disease and possible complications indicate a high level of its social and economic significance [22-25].

Comparison of data on the presence of different groups of contaminants in the samples studied shows that the bacteria of the *Campylobacter* genus are found mainly in raw poultry products and washes from the surfaces of equipment of poultry processing enterprises. A total of 18 isolates of *Campylobacter* from 200 samples containing 15 *C. jejuni* and 3 *C. coli* were isolated. The prevalence of *Campylobacter* spp. in chicken meat and in wash samples was 15.1% and 4.2%, respectively.

Characteristics of the samples from which the *Campylobacter* spp. strains were isolated and identified are given in Table 1. In most cases, *Campylobacter* was isolated from samples contaminated with coliform bacteria (over 60% of samples), while pathogenic Enterobacteria of the *Salmonella* genus were found in 13% of these samples.

The isolated strains of *Campylobacter* according to the main phenotypic features were classified as *C.jejuni* species (87.8%), also 2 strains were identified as *Campylobacter upsaliensis* and *C.lari*, 3 cultures could not be attributed to known *Campylobacter* species, and therefore they were identified as atypical representatives of *Campylobacter* genus.

Table 2 lists the antibiotics used and the availability of antibiotic data in the samples as a percentage. It was found out that the following antibiotics occupied the largest indices in the samples: fluoroquinolone (84%) and macrolide (83%), while nalidixic acid (10%) was the smallest in its turn.

The results of antibiotics' sensitivity and resistance are listed in table 3.

Antibacterial sensitivity indicators of the isolates are listed in tables 3-4.

The results of the antibacterial sensitivity research of 200 *C.jejuni* isolates against 9 different antibacterial agents are listed in table 3.

As a result of studies using the disk diffusion method, it was found that the sensitivity of chicken *C. jejuni* isolates to amoxicillin and streptomycin was 97.0% and 99.0%, respectively; it was also found that all isolates were sensitive to gentamicin. The sensitivity of the chicken isolates to ampicillin, nalidixic acid and tetracycline was 56.0, 78.0 and 48.0%, respectively. It was found out that chicken *C.jejuni* isolates were resistant to one or several antibiotics. Data on the multiple drug resistance of chicken *C.jejuni* isolates are presented in Table 4.

**Table 1 – Characteristics of the samples studied and Campylobacter spp. isolated.**

Sample No.	Isolation source	Presence of indicator and pathogenic contaminants in the sample studied		Campylobacter strain No.	Result of identification of the isolated Campylobacter strain
		E. coli bacteria	Salmonella		
<b>Poultry products</b>					
1	Chicken carcasses frozen	Found in 0,001 g	Not found in 25 g	14 p/1	<i>Campylobacter upsaliensis</i>
2				14 p/2	<i>C.jejuni ssp. jejuni 2</i>
3		14 p/10	<i>C.jejuni ssp. jejuni 2</i>		
4		Not found in 0,01 g	Not found in 25 g	15 p	<i>C.jejuni ssp. jejuni 2</i>
5	Broiler chicken, chilled, of grade 1	Found in 0,001 g	Not found in 25 g	17 p/8	<i>C.jejuni ssp. jejuni 2</i>
6	Chicken skin	Found in 0,001 g	Found in 25 g	9 k	<i>C.jejuni ssp. jejuni 2</i>
7	Chicken skin	Found in 0,001 g	Found in 25 g	10 k	<i>C.jejuni ssp. jejuni 2</i>
8	Chicken skin	Found in 0,001 g	Found in 25 g	11 k	<i>C.jejuni ssp. jejuni 2</i>
9	Chicken skin	No data	Found in 25 g	26 p/16	<i>Campylobacter spp.</i>
10	Broiler chicken liver, chilled	Found in 0,001 g	Not found in 25 g	30 p/5	<i>C.jejuni ssp. jejuni 2</i>
11	Broiler chicken heart, chilled	Found in 0,001 g	Not found in 25 g	1 p/3c	<i>C.jejunissp. doylei</i>
12					<i>C.jejunissp. doylei</i>
13	Broiler chicken lights, chilled	Not found in 0,01 g	Not found in 25 g	31 p/2	<i>C.jejuni ssp. jejuni 2</i>
14				31 p/5	<i>C.jejuni ssp. jejuni 2</i>
15				31 p/6	<i>C.jejuni ssp. jejuni 2</i>
16	Broiler chicken intestine	Found in 0,001 g	Not found in 25 g	1 p/2s	<i>C.jejuni ssp. doylei</i>
17				17 p/8	<i>C.jejuni ssp. jejuni 2</i>
18				32 p/13	<i>C.jejuni ssp. jejuni 2</i>
20	Broiler chicken carcasses, frozen	Found in 0,001 g	Not found in 25 g	32 p/15	<i>C.jejuni ssp. jejuni 2</i>
21				32 p/16	<i>C.jejuni ssp. jejuni 2</i>
22				32 p/17	<i>C.jejuni ssp. jejuni 2</i>
23				32 p/18	<i>C.jejuni ssp. jejuni 2</i>
24				32 p/19	<i>C.jejuni ssp. jejuni 2</i>
25	Chicken carcasses, undrawn	Found in 0,001 g	Not found in 25 g	34 p/1	<i>C.jejuni ssp. jejuni 2</i>
				34 p/2	<i>C.jejuni ssp. jejuni 2</i>
<b>% of positive samples Washes from equipment surfaces</b>					
26	Table for poultry carcasses' dressing	Not found	Not found	1 cm/11	<i>C.jejuni ssp. jejuni</i>
27	Table for poultry carcasses' dressing	Found	Not found	6 cm /7	<i>C.jejuni ssp. jejuni</i>
28	Table for poultry carcasses' sorting	Found	Not found	10 cm /1	<i>C.jejuni ssp. jejuni</i>
29	Table for poultry carcasses' sorting	Found	Found	13 cm /2	<i>C.jejuni ssp. jejuni</i>
30	Slaughter house conveyor	Found	Not found	14 cm /5	<i>C.jejuni ssp. jejuni</i>
31				14 cm /17	<i>C.jejuni ssp. jejuni</i>
32	Saw for semi-carcass	Found	Not found	6 cm /7	<i>C.jejuni ssp. jejuni</i>
33				6 cm /8	
34	Bath for semi-carcass	Found	Not found	2 cm /20	<i>C.jejuni ssp. jejuni</i>
35	Neck cutting machine	Not found	Not found	20 cm /2	<i>C.jejuni ssp. jejuni</i>
36		Found	Not found	20 cm /3	<i>C.jejuni ssp. jejuni</i>
37	Stomach cleaning machine	Found	Not found	20 cm /5	<i>C.jejuni ssp. jejuni</i>
38				20 cm /6	<i>C.jejuni ssp. jejuni</i>
39	Table for poultry carcasses drawing	Found	Not found	33 cm /2	<i>C.jejuni ssp. jejuni</i>
40				<i>C.jejuni ssp. jejuni</i>	
41	Blood bath	Found	Not found	15 cm /2	<i>C.jejuni ssp. jejuni</i>
42				15 cm /3	<i>C.lari</i>
43				15 cm /4	<i>C.jejuni ssp. jejuni</i>
44				15 cm /5	<i>C.jejuni ssp. jejuni</i>
45				16 cm /1	<i>Campylobacter spp.</i>
46	Blood bath	Found	Not found	16 cm /6	<i>C.jejuni ssp. jejuni</i>
47				16 cm /7	<i>C.jejuni ssp. jejuni</i>
48				16 cm /10	<i>C.jejuni ssp. jejuni</i>
49				17 cm /1	<i>Campylobacter spp.</i>
50	Feather boxes	Found	Not found	17 cm /7	<i>C.jejuni ssp. jejuni</i>

**Table 2 – List of the antibiotics used**

No.	Antibiotics used	Presence in samples
1	ampicillin	48.8%
2	amoxicillin	84%
3	gentamicin	83%
4	nalidixic acid	10%
5	streptomycin	31%
6	tetracycline	79%
7	penicillin	33%
8	erythromycin	67.2%
9	ciprofloxacin	62.1%

**Table 3 – Indicators of antibacterial resistance and distribution of minimum inhibitory concentration of chicken C.jejuni isolates.**

Antibiotics	Chicken (n = 100)		
	sensitive	rmedium-resistan	resistant
ampicillin	56	12	32
amoxicillin	97	2	1
gentamicin	100	–	–
nalidixic acid	78	3	19
streptomycin	99	–	1
tetracycline	48	6	56
penicillin	56	9	35
erythromycin	67	4	29
ciprofloxacin	69	12	19

**Table 4 – Distribution of multiple drug resistance of C.jejuni isolates.**

Number of resistance	Models of resistance	Origin of resistant isolates
		Chicken
2	AMP, EM	1
2	AMP, NA	1
3	NA, S	1
4	AMC, AMP, TE	–
4	AMP, NA, TE.	1
4	AMP, EM, NA	4
5	AMC, AMP, EM, NA	1
5	AMP, EM, NA	1
5	AMP, NA, TE	12
5	EM, NA, TE	2
5	NA, S, TE	–

Similar results were also noted by the following investigators. Han et al. (2007) found that all isolates were resistant to at least one antibacterial agent, whereas most isolates were resistant to tetracycline, nalidixic acid and ciprofloxacin. In addition, most of them were sensitive to erythromycin, chloramphenicol and gentamicin.

Zhang et al. (2010) announced that 44 C. Jejuni isolates were sensitive to erythromycin, gentamicin and streptomycin, but resistant to nalidixic acid, levofloxacin and ciprofloxacin. In the research conducted in the Czech Republic by Steinhäuserová and Mikulíková (2005), as a result of testing antibacterial sensitivity of poultry and human isolates, C.jejuni proved to be resistant to ciprofloxacin within 59% and 69%, and two groups of isolates were resistant to nalidixic acid within 30%. Rahimi et al. (2010) found that resistance indicators of 177 C.jejuni strains isolated from poultry carcasses to tetracycline, ciprofloxacin, nalidixic acid and enrofloxacin were 79.7%, 67.2%, 59.3% and 48%,

respectively. Moreover, researchers reported that 14.3% of isolates were resistant to one antibiotic, 24.3% of isolates were resistant to two antibiotics, and 40% of isolates were resistant to more than two antibiotics. High level of C.jejuni resistance to ciprofloxacin (80%) was found in 117 human and 33 poultry isolates, according to data of Senok et al. (2007), in Sudan. Besides, all the isolates were sensitive to erythromycin except two human isolates. Kos et al. (2006) noted that 104 C.jejuni isolates isolated from poultry carcasses were resistant to tetracycline, nalidixic acid and ciprofloxacin with the indicators of 69%, 11% and 8%, respectively; they also found that all the isolates were sensitive to gentamicin and erythromycin. Mifflin et al. (2007) showed that in disk diffusion test, 125 C.jejuni isolates from broiler chicken carcasses proved to be resistant to tetracycline and ampicillin with the indicators of 18.4% and 17.6%, respectively, and all the isolates were sensitive to erythromycin. Wiczorek et al. (2012) noted that in Poland, 122 C.jejuni strains isolated from chicken meat were resistant to ciprofloxacin, nalidixic acid and tetracycline with the indicators of 91%, 89.3% and 49.1%, respectively; and all the isolates were sensitive to erythromycin and gentamicin [19, 21-35].

As a result of our study, taking into account the high rates of multiple antibiotic resistances, there was a low level of accordance with the results of genotyping and resistance to antibiotics. Similar data were obtained by Khan et al. (2007) and other researchers. Thus, the relationship between the genotypic characteristic and resistance to antibiotics was rather low.

#### CONCLUSION

Phenotypic profiles of antibiotic resistance of 9 C. *Jejuni* strains isolated from raw poultry products and washes from equipment surfaces of poultry processing enterprises were studied.

Sensitivity of the isolated strains to 9 antibiotics was defined. None of the isolates was resistant to amoxicillin, gentamicin and streptomycin. At the same time a different pattern of resistance was observed for other antibiotics: tetracycline, erythromycin and nalidixic acid, respectively. Obtained data prove high prevalence of antibiotic-resistant strains among campylobacteria contaminating poultry products during production and processing of raw material.

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