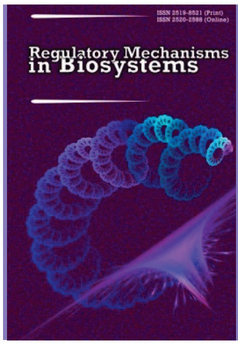

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Phenotypic patterns of antimicrobial resistance in *Campylobacter* spp. in Ukraine

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Campylobacter spp. are major foodborne zoonotic pathogens that have recently become more resistant to fluoroquinolones and macrolides, which are broad-spectrum antibiotics used in both medicine and veterinary practice. *Campylobacter* is a commensal of the intestines of mammals and birds, which facilitates the transfer of antimicrobial resistance (AMR) determinants from other bacteria through horizontal gene transfer. The aim of this study was to establish the prevalence and determine the AMR phenotypes of *Campylobacter* species isolated in Ukraine. Using the disk diffusion method (DDM), 33 isolates of *Campylobacter* spp. isolated from animals and poultry on farms between May and September 2023 were tested. Additionally, an analysis of the resistance of 293 *Campylobacter* spp. isolates obtained from children with acute intestinal infections from 2020 to 2023 was conducted. The level of resistance of clinical *Campylobacter* spp. to ciprofloxacin (CIP) was found to be 83.3%, to tetracycline (TE) 53.6%, to erythromycin (E) 11.6%, and the isolates from farms showed resistance to ciprofloxacin (CIP) at 72.7%, to tetracycline (TE) at 60.6%, and to erythromycin (E) at 18.2%. In 4.1% of clinical isolates, multidrug resistance (MDR) (CIP/TE/E) was detected, with the most common AMR combination being (CIP/TE), reaching 42.7%. Resistance to at least one antibiotic was found in 37.8%. The isolates from animals and poultry had a multiple antibiotic resistance rate of 12.1%, with 42.4% being resistant to at least one antibiotic. This study provides insight into the relevance and importance of *Campylobacter* spp. resistance in Ukraine. It expands the understanding of the issue, which requires more detailed study, including the molecular mechanisms of resistance and the identification of genetic determinants shaping the epidemiology of antimicrobial resistance in Ukraine. For this purpose, a collection of isolates has been created, and optimal long-term storage conditions have been selected, which will allow the study of *Campylobacter* spp. decades from now.

Keywords: foodborne pathogens of Ukraine; children; poultry; livestock; multidrug resistance; lyophilised culture.

Introduction

Campylobacter spp., especially the thermophilic species *Campylobacter jejuni* and *Campylobacter coli*, are major food-borne zoonotic pathogens, responsible for 400–500 million cases of gastrointestinal infections annually due to consumption of contaminated food of animal origin or contact with sick animals (Igwaran & Okoh, 2019). In the gut microbiota of many animals and poultry, *Campylobacter* is a commensal, with chicken considered the main natural source of *Campylobacter* infection (Bolton, 2015; Enany et al., 2021). The course of campylobacteriosis is characterized by acute diarrhea that does not require antimicrobial treatment, as it is short-term and self-limited, but for severe and prolonged infections or for extraintestinal post-infectious complications, treatment with antibiotics such as macrolides and fluoroquinolones is necessary (Sproston et al., 2018).

Excessive consumption of antibiotics in medicine and dangerously uncontrolled use in livestock husbandry to stimulate growth, prevent and treat bacterial infections is accompanied by the emergence of resistant zoonotic pathogens, which leads to a decrease in the effectiveness of medicines in treatment. The constant exposure to low-dose antibiotics creates selective pressure on bacterial pathogen populations, leading to the adaptation and spread of resistant and multidrug-resistant strains. This contributes to the spread of antibiotic resistance among animals, in the environment and through the food chain to humans (Salmanov & Muzyka 2017). The rise of antibiotic resistance is a global public health threat (Tang et al., 2023). As a foodborne pathogen and constantly exposed to antibiotics, *Campylobacter* has evolved mechanisms of antimicrobial resistance (Shen et al., 2018). According to the reports of the European Food Safety Agency (EFSA) and the European Agency for Safety and Disease Control (ECDC), in 2022, resistance to ciprofloxacin of *Campylobacter jejuni* isolates from humans reached 96.6% and from broilers – 70.9%. For many years, highly effective antibiotics for the treatment of campylobacteriosis were fluoroquinolones, but their use in animal husbandry as growth promoters created the basis for the emergence of fluoroquinolone-resistant *Campylobacter* (Portes et al., 2023). Due to widespread resistance to fluoroquinolones, WHO has included *Campylobacter* spp. to the list of priority pathogens for which the development of new antibiotics is urgently needed (Zhang et al., 2023).

The presence of resistance genes in *Campylobacter* that can spread resistance to a wide range of antibiotics through horizontal transfer both within the genus and with other bacteria is a growing concern (Rivera-Mendoza et al., 2020; Bunduruş et al., 2023). All this requires systematic research, control and surveillance of *Campylobacter* with constant sensitivity testing (Bunduruş et al., 2023).

The USA, EU and Australia/New Zealand regulations are considered to be the most advanced control system for *Campylobacter* in animal products (Portes et al., 2023). Ukraine has no control levers for *Campylobacter* at any stage of the food chain. The lack of studies of *Campylobacter* on a permanent basis as a food pathogen, the high cost of diagnostic work and the low level of bacteriological diagnosis, which is associated with the fastidiousness of the cultivation conditions and environment, delay the study of the zoonotic pathogen in Ukraine (Pinchuk & Pustovit, 2018; Shchur et al., 2023; Shchur et al., 2024).

Monitoring of *Campylobacter* in poultry farming, according to the principle of the nationwide program to combat salmonellosis in poultry pursuant to the "Instructions for the Prevention and Elimination of Salmonellosis in Poultry" in accordance with Order No. 310 approved by the Ministry of Agrarian Policy and Food of Ukraine dated September 19, 2016, is not available in Ukraine. For the first time and so far only once, in 2021, was "State monitoring of antimicrobial resistance in veterinary medicine" conducted, within the framework of the State Strategy of Ukraine on curbing antimicrobial resistance and reducing the risks of formation and spread of antibiotic-resistant microorganisms in livestock. A total of 448 isolates of pathogenic and conditionally pathogenic microorganisms were isolated, of which 7.4% were *Campylobacter* spp. against 4.2% *Salmonella* spp. The widespread distribution of *Campylobacter* isolates among animals and poultry, as well as in farms with industrial and traditional technologies of keeping in the territories of Dnipropetrovsk, Lviv, Cherkasy, Chernihiv, Kherson and Khmelnytsky regions, was revealed. This work established phenotypes of antibiotic resistance and identified multiresistant *Campylobacter* spp. from poultry, which was 42.4% (Mazur et al., 2022; Chechet et al., 2023; Shchur et al., 2023).

Therefore, the purpose of our study was to show the frequency of isolation of *Campylobacter* spp., distribution, species diversity, to investigate the resistance of *Campylobacter* to antibiotics and to analyze the phenotypic resistance profiles.

Materials and methods

Place of research. The studies were conducted on the basis of the research bacteriological department of the State Scientific Research Institute for Laboratory Diagnostics and Veterinary Sanitary Examination (SSRILDVSE) Kyiv, Ukraine.

Collection of *Campylobacter* spp. isolates from livestock and poultry. Livestock and poultry were slaughtered in slaughterhouses in compliance with standard operating procedures for livestock welfare during slaughter in accordance with EU Council Directive 1099/2009, EU Council Directive 98/58 applicable to all livestock reared or kept for food production and EU Council Directive 1/2025 on the protection of livestock in transit (Nedosekov & Petkun, 2021; Petkun & Nedosekov, 2022). The selection of pathological material was carried out by specialists familiar with the rules of selection and transportation to the laboratory. In accordance with the initiative research topic 0118U100599 "Assessment of the degree of spread of antibiotic resistance of bacterial zoonoses in Ukraine", during May–September 2023, 33 isolates of *Campylobacter* spp. were collected. from animals and poultry. The isolates were taken from cattle 3.0% (1/33), pigs 33.4% (11/33), broiler chickens 60.6% (20/33), turkeys 3.0% (1/33) from different natural and geographical regions of Ukraine. The western part of Ukraine is represented by Volyn, the northern part by Kyiv, and the central part by Vinnytsia and Cherkasy regions.

Collection of data on isolates of *Campylobacter* spp. from children. Pursuant to the order of the Ministry of Health of Ukraine No. 905, according to Decision No. 2119/EC of the European Parliament and Council, according to the established criteria for determining cases of campylobacteriosis, during the years 2020–2023, 293 isolates from pediatric patients of the infectious department of the "City Clinical Hospital" No. 9" of the city of Dnipro were isolated and tested for sensitivity to antibiotics According to the Agreement No. 89 on cooperation and organization of relations between the NULES of Ukraine and the "City Clinical Hospital No. 9" of the city of Dnipro, we received reported data on the types and sensitivity to antimicrobial drugs of isolated isolates of *Campylobacter* spp. from children. The criterion for the selection of reported data was the methodological approach to the treatment protocols of "Acute intestinal infection" with bacteriological detection of *Campylobacter* spp. in biological samples.

Detection of Campylobacter spp. from children. The laboratory for detection and identification of *Campylobacter* spp. uses "Basic laboratory procedures in clinical bacteriology" (Vandepitte, 2003). Isolation was carried out on plates with *Campylobacter* Agar Base (M994) (HiMedia, India, 2025) with a mixture of antibiotics (FD006) (HiMedia, India, 2024) and the addition of 5% Horse Blood Lysed (HB037) (TCS Biosciences Ltd, United Kingdom, 2024). The isolates were incubated at 42 °C for 48 hours under a microaerobic atmosphere (5% O_2, 10% CO_2, and 85% N_2) created using GENbox microaer gas-generating bags (BioMerieux, France, 2024). After identifying typical colonies, Gram staining, microscopy and determination of generic and species properties using biochemical tests was carried out:

– test for the ability to produce cytochrome oxidase;

– test for the ability to produce catalase;

– determination of mobility in a "crushed drop";

– temperature test (ability of the culture to grow on *Campylobacter* Agar under microaerobic conditions at 25degrees and 37degrees);

– growth test on Olkenitsky agar.

Definition of *Campylobacter* spp. species:

– test for the ability to hydrolyze sodium hippurate;

– test for resistance to nalidixic acid.

Detection of Campylobacter spp. from livestock and poultry samples. Detection and identification were based on ISO 10272-1:2017(E) microbiology of the food chain – Horizontal method for detection and enumeration of *Campylobacter* spp. – Part 1: Detection method (ISO 10272-1:2017(E). Isolation was carried out on plates with Blood Free *Campylobacter* Selectivity Agar Base – mCCD agar (M887) (HiMedia, India, 2024) with the addition of *Campylobacter* Supplement V (BFCSA) (FD067) (HiMedia, India, 2023). Incubated at a temperature of 41.5 °C for 48 hours in a microaerobic atmosphere, which was created using gas-generating packages GENbox microaer (VioMerieux, France, 2024). Typical colonies were subjected to Gram staining and microscopy. Morphological (thin spirally bent Gram-negative rods) and biochemical (testing for oxidase and catalase) properties were determined. The selected isolates were identified to the species level by the MALDI-TOF MS method using a VITEK analyzer (VioMerieux, France).

Determination of sensitivity to antibiotics. For sensitivity testing of isolates of *Campylobacter* spp. for antibacterial drugs, the Kirby-Bauer disc-diffusion method (DDM) was used using Mueller Hinton Agar (M173) (HiMedia, India, 2026) with the addition of 5% Horse Blood Lysed (HB037) (TCS Biosciences Ltd, United Kingdom, 2024). Preparation of cups with medium was carried out according to the manufacturer's instructions. Bacterial suspension was prepared by adding *Campylobacter* culture to sterile physiological solution and adjusted to an optical density of 0.5 according to the McFarland standard (R092) scale (HiMedia, India, 2024). Three groups of antibiotics were studied according to the Guidelines of the European Committee for Antimicrobial Susceptibility Testing – EUCAST. Antibiotic discs were used in concentration: ciprofloxacin 5 µg (CIP) (SD060), tetracycline 30 µg (TE) (SD037) and erythromycin 15 µg (E) (SD013) (HiMedia, India, 2026). The suspension from each isolate was placed in a dish with agar and uniformly inoculated with a sterile cotton swab over the entire surface of the medium. After that, discs with antibiotics were applied and incubated at a temperature of 41.5 °C for 24–48 hours in microaerobic conditions. The diameters of the zones of growth retardation around the disc with the antibiotic were measured with a ruler from (HiMedia, India). Antibiotic susceptibility of *Campylobacter* isolates was assessed according to EUCAST recommendations (www.eucast.org/clinical_breakpoints). Research quality control was performed with the reference museum strain *Campylobacter jejuni* ATCC 33291.

The creation of a Campylobacter spp. isolate collection. To study the epidemiology of campylobacteriosis, as well as the biological and molecular-genetic properties of the pathogens of this zoonosis, it is necessary to create a collection of field isolates of *Campylobacter*. The purpose of creating this collection is the need to preserve contemporary *Campylobacter* isolates for future research using methods that will be developed in the future.

The research was based on isolates we obtained from animals and poultry in various natural-geographical regions of Ukraine. The registration and accounting of the isolates were conducted using a system that included: the date the sample was received, the type of animal/poultry, the type of sample, the place and date of collection, the date the isolate was obtained, and identification.

To systematize the obtained isolates, a unified registration system was developed according to the proposed designations. Each isolate was assigned an identification number consisting of the last two digits of the year of isolation from pathological/biological material, the designation of the animal/poultry species in Latin letters (CA – cattle, PI – pig, BR – broiler, TU – turkey, H – human), and the serial number in the registration journal.

The code 23TU8 indicates material collected in 2023 from a turkey, which is recorded in the registration journal under number 8.

Lyophilization of the obtained Campylobacter spp. for long-term storage. Culture media were prepared and controlled according to ISO 11133:2014 "Microbiology of food, animal feed and water – Preparation, production, storage and performance testing of culture media." Faybich's medium (gelatin – 1.0%, sucrose – 10.0%, distilled water – 89.0%) was used as a cryoprotectant. The quality of the distilled water was controlled according to DSTU ISO 3696:2003 "Water for laboratory use. Requirements and test methods." A protective medium was added to the 24-hour broth culture of *Campylobacter* in a 1:1 volume ratio. The mixture was then distributed into 5 cm³ vials at a volume of 1.0 cm³. Before lyophilization (cryopreservation and subsequent sublimation), a vial from each batch was taken to determine the typicality, uniformity, and concentration of bacterial mass (CFU/cm³). Cryopreservation of the isolates was carried out in an "Arctiko LF 100" freezer (Denmark) at a temperature of –70.0 ± 5.0 °C. Sublimation drying was performed in a Telstar Qriodoz apparatus at a condenser temperature of –45.0 ± 5.0 °C, a vacuum depth of 0.15–0.17 mBar, for 24 hours. After drying, the number of colony-forming units of *Campylobacter* (CFU/cm³) was counted according to EN ISO 10272 – Microbiology of the food chain – Horizontal method for detection and enumeration of *Campylobacter* spp. – Part 2: Colony-count technique. The lyophilized dehydrated suspension in vials was rehydrated to the original volumes using Nutrient Broth (M002) (HiMedia, India, 2026). Plates with mCCD agar (M887) (HiMedia, India, 2024) were inoculated. The cultures were incubated at 42 °C in microaerobic conditions for 48 hours.

Results

Identification of Campylobacter spp. isolates. We examined 138 samples of the contents of cecums/cecal appendices from animals and poultry collected during slaughter. From these, 33 *Campylobacter* spp. isolates were identified, representing 23.9% of the lesions. Of these isolates, 36.4% were from animals (cattle and pigs) and 63.6% from poultry (broiler chickens and turkeys). All isolates were subjected to species identification. Among them, *Campylobacter jejuni* accounted for 24.2% (8/33) and *Campylobacter coli* for 75.8% (25/33) (Table 1).

Table 1
Spectrum of *Campylobacter* isolated from livestock and poultry in Ukraine in 2023 (N = 33)

Samples	*Campylobacter jejuni*		*Campylobacter coli*		Total	
	number	%, of the total	number	%, of the total	number	%, of the total
Livestock	1	3.0	11	33.4	12	36.4
Poultry	7	21.2	14	42.4	21	63.6
Total	8	24.2	25	75.8	33	100

Analyzing the report data from the bacteriological laboratory of "City Clinical Hospital No. 9" for the period from 2020 to 2023, it was established that 6,055 biological samples from children with acute gastrointestinal infections (AGI) were examined. Among these, 293 *Campylobacter* spp. isolates were identified, representing 4.8% of the lesions. It was found that *Campylobacter jejuni* occupies the largest proportion of isolates and accounts for 55.3% (162/293). This is followed by *Campylobacter coli* – 37.2% (109/293) and *Campylobacter lari* – 7.5% (22/293) (Table 2).

Analysis of susceptibility to antimicrobial drugs of isolates of Campylobacter spp. from children. Campylobacter was found to be largely resistant to fluoroquinolones 83.3% (244/293) and tetracyclines 53.6% (157/293). *Campylobacter lari* isolates had the highest resistance to ciprofloxacin – 100.0% (22/22). *Campylobacter coli* demonstrated higher resistance to macrolides than *Campylobacter jejuni*, which was 16.5% (18/109) versus 9.3% (15/162). One isolate of *Campylobacter lari* with acquired resistance to erythromycin was detected – 4.5% (1/22) (Table 3).

The most common antimicrobial resistance phenotype of *Campylobacter jejuni* isolates was CIP/TE, which accounted for 46.3%. Multidrug-resistant isolates were found in 1.2% of isolates, resistant to at least one antibiotic – in 37.7%, and sensitive to all tested antibiotics were 8.0% of *Campylobacter jejuni*.

Resistance to two antibiotics was detected in 39.4% (43/109) of *Campylobacter coli*, the most common combination being CIP/TE – 32.1%. Multidrug resistance reached 8.3%, but there were also 11.9% of sensitive isolates among *Campylobacter coli*. Resistant to at least one antibiotic were 40.4%. Among *Campylobacter lari*, 1 multidrug-resistant isolate was detected, 27.3% were resistant to a single antibiotic (ciprofloxacin). No sensitive isolates were detected. Two antibiotics (CIP/TE) were resistant in 68.2% of isolates (Table 4).

Table 2
Spectrum of *Campylobacter* isolated from children in Ukraine during 2020–2023 (N = 293)

Year	*Campylobacter jejuni*		*Campylobacter coli*		*Campylobacter lari*		Total	
	number	%, of the total	number	%, of the total	number	%, of the total	number	%, of the total
2020	55	57.3	36	37.5	5	5.2	96	32.8
2021	46	57.5	30	37.5	4	5.0	80	27.3
2022	26	44.1	24	40.7	9	15.2	59	20.1
2023	35	60.3	19	32.8	4	6.9	58	19.8
Total	162	55.3	109	37.2	22	7.5	293	100

Table 3
Resistance of *Campylobacter* spp. isolates from children (N = 293)

Antibiotics	*Campylobacter jejuni*		*Campylobacter coli*		*Campylobacter lari*		Total	
	number	%, of the total	number	%, of the total	number	%, of the total	number	%, of the total
Ciprofloxacin (5 µg)	137	84.6	85	78.0	22	100	244	83.3
Erythromycin (15 µg)	15	9.3	18	16.5	1	4.5	34	11.6
Tetracycline (30 µg)	87	53.7	54	49.5	16	72.7	157	53.6

Analyzing the resistance of *Campylobacter* by year, there is a noticeable increase in resistance to ciprofloxacin, from 73% in 2020 to 98% in 2023. Resistance to tetracycline also shows an upward trend, increasing from 55% in 2020 to 66% in 2023. Resistance to erythromycin demonstrates fluctuations, ranging from 8% in 2020 to 17% in 2021, then decreasing to 5% in 2022, and rising again to 16% in 2023 (Table 5).

Antimicrobial susceptibility testing of Campylobacter spp. isolates from animals and poultry. The majority of isolated isolates from animals and poultry showed resistance to ciprofloxacin 72.7% (24/33) and tetracycline 60.6% (20/33).

Of the studied isolates of *Campylobacter coli* from pigs, 63.6% (7/11) had resistance to ciprofloxacin and 72.7% (8/11) – to tetracycline.

The resistance to ciprofloxacin of isolates from poultry was 76.2% (16/21) and to erythromycin – 19.0% (4/21). The *Campylobacter jejuni* isolate from cattle was resistant to ciprofloxacin and tetracycline, and was sensitive to erythromycin (Table 6).

Established phenotypes of antimicrobial resistance of *Campylobacter jejuni* isolates from broilers: CIP/TE/E – 16.7% (1/6), CIP/E – 16.7% (1/6), 66.6% (4/6) were resistant to one of three antimicrobial drugs, isolates sensitive to all three antibiotics were not found.

Table 4
Antimicrobial resistance phenotypes of *Campylobacter* spp. isolates in children

Antibiotic resistance phenotypes	*Campylobacter jejuni*		*Campylobacter coli*		*Campylobacter lari*		Total	
	number	%, of the total	number	%, of the total	number	%, of the total	number	%, of the total
CIP/TE/E	2	1.2	9	8.3	1	4.5	12	4.1
CIP/TE	75	46.3	35	32.1	15	68.2	125	42.7
CIP/E	5	3.1	4	3.7	0	0	9	3.1
TE/E	6	3.7	4	3.7	0	0	10	3.4
CIP	55	33.9	37	33.9	6	27.3	98	33.4
TE	4	2.5	6	5.5	0	0	10	3.4
E	2	1.3	1	0.9	0	0	3	1.0
SENSITIVE	13	8.0	13	11.9	0	0	26	8.9

Table 5
Number of *Campylobacter* spp. strains isolated from children and their antibiotic susceptibility in 2020–2023 (N = 293)

Year	*Campylobacter* spp.	Ciprofloxacin (5 µg)		Erythromycin (15 µg)		Tetracycline (30 µg)	
	number	%, sensitive	%, resistant	%, sensitive	%, resistant	%, sensitive	%, resistant
2020	96	27	73	92	8	45	55
2021	80	20	80	83	17	54	46
2022	59	10	90	95	5	51	49
2023	58	2	98	84	16	34	66
Total	293	16.7	83.3	88.4	11.6	46.4	53.6

Table 6
Resistance of *Campylobacter* spp. isolates from livestock and poultry

Antibiotics	Livestock		Poultry		Total	
	number	%, of the total	number	%, of the total	number	%, of the total
Ciprofloxacin (5 µg)	8	66.7	16	76.2	24	72.7
Erythromycin (15 µg)	2	16.7	4	19.0	6	18.2
Tetracycline (30 µg)	9	75.0	11	52.4	20	60.6

Table 7
Antimicrobial resistance phenotypes of *Campylobacter* spp. isolates from livestock and poultry

Antibiotic resistance phenotypes	*Campylobacter jejuni*		*Campylobacter coli*		Total	
	number	%, of the total	number	%, of the total	number	%, of the total
CIP/TE/E	2	25.0	2	8.0	4	12.1
CIP/TE	1	12.5	9	36.0	10	30.3
CIP/E	1	12.5	0	0	1	3.0
TE/E	0	0	1	4.0	1	3.0
CIP	2	25.0	7	28.0	9	27.3
TE	2	25.0	3	12.0	5	15.2
E	0	0	0	0	0	0
SENSITIVE	0	0	3	12.0	3	9.1

Phenotypes of antimicrobial resistance of *Campylobacter coli* isolates from broilers: CIP/TE/E – 7.1% (1/14), CIP/TE – 35.7% (5/14), 42.8% (6/14) isolates, were resistant to one of three antimicrobial drugs, 14.3% (2/14) were.not resistant to all three antibiotics.

The isolate of *Campylobacter jejuni* from turkeys had multiresistance to the tested antibiotics.

Phenotypes of antimicrobial resistance of *Campylobacter coli* isolates from pigs were established. The CIP/TE combination was detected in 36.3% (4/11), TE/E in 9.1% (1/11), CIP/TE/E multiresistance was found in 9.1% (1/11). 36.4% (4/11) were resistant to one antibiotic, 9.1% (1/11) were sensitive to all tested groups of antimicrobials.

Among *Campylobacter coli*, the most common combination of antibiotic resistance was CIP/TE. Both multidrug-resistant and sensitive isolates were detected. Among *Campylobacter jejuni*, no sensitive isolates were detected, and both multidrug-resistant and dual antibiotic-resistant isolates prevailed (Table 7).

Collection of lyophilized cultures. We assessed the uniformity, homogeneity, and the number of colony-forming units (CFU/cm³) of *Campylobacter* before and after lyophilization. For this, the seeded material was examined on plates with a dense nutrient medium, mCCD agar. The uniformly seeded surface of the agar was noted for colonies with a shiny surface, greyish colour with a metallic sheen (Fig. 1). Microscopic preparations were made and stained using the Gram method. Gram-negative, thin, spiral-shaped bacteria resembling "comma" or "seagull wings" were observed in the smears (Fig. 2). The quality of the lyophilized cultures was evaluated based on their survival rate before and after lyophilization. The activity of *Campylobacter* was determined by the number of microorganisms per 1 cm³. This involved plating appropriate serial dilutions in sterile physiological saline onto mCCD agar. The viability of *Campylobacter* before lyophilization was 8.0×10^7 CFU/cm³, while after lyophilization it was 6.0×10^5 CFU/cm³. After the lyophilization process, the number of viable microbial cells was 0.75% of the initial level.

The lyophilized culture collection was stored at a temperature of –18 ± 2 °C. The lyophilized *Campylobacter coli* culture was revived at 1, 3, 6, 9, and 12 months. The results showed that the viability of *Campylobacter* was 0.73% of the initial level after 1 month, 0.68% after 3 months, 0.65% after 6 months, 0.63% after 9 months, and 0.6% after 12 months. After one year of storage following lyophilization, *Campylobacter coli* (23BR7) regained 80% of its viability.

Discussion

Resistance of microorganisms to antimicrobial drugs is a global threat to "One Health". Foodborne pathogens with multidrug resistance pose serious challenges to the population in terms of consuming safe and quality food products of animal origin (Rafiq et al., 2022). Thermophilic bacteria of the genus *Campylobacter* are the most common food-borne zoonotic pathogens, so their resistance to antimicrobial drugs is a public health problem, and multiresistance becomes a potential danger and requires an urgent response (Zhang et al., 2023).

Fig. 1. Colonies of *Campylobacter* spp. on mCCD agar: rounded with smooth edges, shiny convex surface, greyish in colour with a metallic sheen; with the ability to form a plaque on the surface of the medium (the 'swarming' effect)

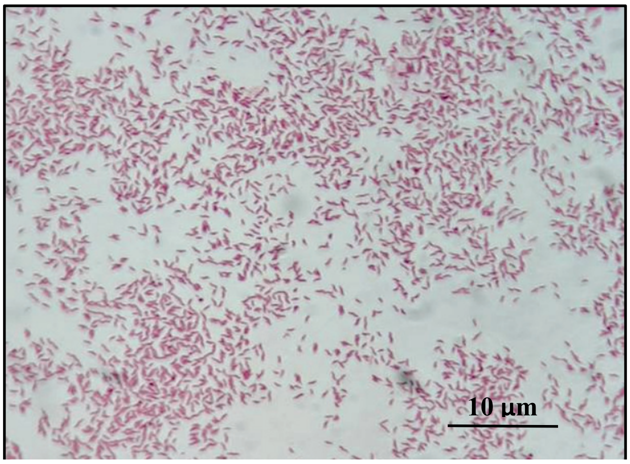

Fig. 2. *Campylobacter* under the microscope, fuchsin staining

In the European Union, campylobacteriosis overtook salmonellosis for the first time in 2005 and remains the most common intestinal infection among humans (Iovine, 2013). According to the latest reports of the Central Health Center of the Ministry of Health of Ukraine on cases of campylobacteriosis, the average annual incidence rate from 2016 to 2023 is 0.34 cases per 100,000 population (Public Health Center of Ukraine, 2024). Although about 4.0% of campylobacteriosis cases in children are registered annually in one of the Dnipro hospitals, the most vulnerable are children under one year – 14% and from 1 to 3 years – 49%. Similar results were obtained during epidemiological surveillance during bacteriological studies on *Campylobacter* materials from children with acute intestinal infections. In Kyiv hospitals, the disease was 2.9%, Dnipropetrovsk – 2.1%, Zaporizhia – 3.4%, Cherkasy – 1.1% (Kyryk, 2013; Kyryk, 2017). The difference between official data on campylobacteriosis and real results indicates the imperfect diagnosis of *Campylobacter* in Ukraine (Kyryk, 2012). This is confirmed by the cases of GKI and food toxic infections of unknown etiology, which in 2023 amounted to more than 70 cases per 100,000 people (Public Health Center of Ukraine. Monitoring and Statistics [Internet]. Kyiv: Public Health Center of the Ministry of Health of Ukraine; 2024 [updated 2024 Jan; cited 2024 Jan 21] Available from: https://phc.org.ua/kontrol-zakhvoryuvan/inshi-infekciyni-zakhvoryuvannya/infekciyna-zakhvoryuvanist-naselennya-ukraini).

Campylobacteriosis of livestock and poultry is almost never registered in Ukraine (Tsarenko & Korniienko, 2021). Thus, for the period from 2009 to 2023, 11 positive results were obtained without species identification for studies of aborted fetuses from pigs and cattle, and no positive results were obtained for studies of materials from poultry ("Report on the work of laboratories of the State Production and Consumer Service of Ukraine" No. 2-Vet). The exception is isolated research studies (Shchur et al., 2024).

Campylobacter jejuni and *Campylobacter coli* are the most common species that cause bacterial foodborne infections (Hansson et al., 2018). In our study, the most common causative agent of campylobacteriosis in children was *Campylobacter jejuni* 55.3% (162/293), and the second – *Campylobacter coli* 37.2% (109/293), which is consistent with the results of other studies (Metreveli et al., 2022; Mulu et al., 2024). Among isolates isolated from broiler chickens, *Campylobacter coli* prevailed – 70.0% (14/20), similar results were obtained in Ecuador, China and the United Arab Emirates (Vinueza-Burgos et al., 2017; Zhang et al., 2018; Habib et al., 2022), although *Campylobacter jejuni* is most commonly found in chickens (Hafez et al., 2018; Ortiz et al., 2024).

The intensive use of fluoroquinolones in poultry farming has contributed to the emergence and spread of resistant *Campylobacter jejuni*. Since the first reports of *Campylobacter* resistance to fluoroquinolones in the late 1980s, resistance has continued to increase (Iovine, 2013; Veltcheva et al., 2022). Persistent resistance of *Campylobacter* to fluoroquinolones was found in Korea, China, Turkey (Kim et al., 2016; Tang et al., 2020; Eryıldız et al., 2022), several countries of South America and the USA (Portes et al., 2023; Yan et al., 2023). In our study, resistance to ciprofloxacin was found in clinical isolates of *Campylobacter jejuni* 84.6% (137/162), isolates of *Campylobacter coli* 78.0% (85/109) and isolates of *Campylobacter lari* 100% (22/22). The resistance of *Campylobacter coli* isolates from pigs is 63.6% (7/11) and from broilers is 78.6% (11/14).

The most commonly used antibiotic in the macrolide group is erythromycin, which is considered the drug of choice for the treatment of *Campylobacter* infections. *Campylobacter* was thought to be genetically resistant to erythromycin, but in 2014, transmissible plasmid resistance to erythromycin was first described (Wang et al., 2014). *Campylobacter* resistance to macrolides is lower than resistance to fluoroquinolones. It is interesting that *Campylobacter coli* shows higher resistance to antibiotics of this group than *Campylobacter jejuni*. According to EFSA & ECDC reports, isolates of *Campylobacter jejuni* from humans and animals had very low or no resistance. The resistance of *Campylobacter coli* from humans reached the level of 7.8%, from cattle under one year of age – 35.7%, which is consistent with the results of our research. During our study, clinical isolates of *Campylobacter jejuni* had resistance to erythromycin 9.3% (15/162), isolates from cattle did not show resistance. *Campylobacter jejuni* isolates from poultry were 42.8% resistant (3/7). *Campylobacter coli* isolates had higher resistance. Thus, resistance reached 16.5% (18/109) from children, 7.1% (1/14) from poultry, and 18.2% (2/11) from pigs. High resistance of *Campylobacter coli* to macrolides was obtained in Korea, Egypt, China (Choi et al., 2021; Mouftah et al., 2021; Zhang et al., 2022).

Tetracyclines are widely used in the prevention and treatment of infections in animals and humans. The low cost and broad spectrum of antimicrobial activity of tetracycline makes it suitable for use in subtherapeutic doses as a growth promoter in animal feed (Hormeño et al., 2020). As a result of our research, the resistance of clinical isolates of *Campylobacter jejuni* 53.7% (87/162), *Campylobacter coli* 49.5% (54/109) and *Campylobacter lari* 72.7% (16/22) was revealed. The obtained resistance of *Campylobacter coli* from pigs is 72.7% (8/11) and from poultry 50.0% (7/14). Resistance to tetracycline in clinical isolates from patients with campylobacteriosis in the United States was higher than resistance to ciprofloxacin and amounted to 51.6% (Rodrigues et al., 2021). Results with persistent resistance of *Campylobacter coli* isolates from poultry were obtained in Peru (Benites et al., 2022).

Multi-resistance (MDR) to three antibiotics of *Campylobacter jejuni* from humans in the EU was at a low level of 0.7%. *Campylobacter coli* had higher resistance: from humans – 9.0%, broilers – 8.3%, cattle – 39.3%, turkeys – 16.9%, pigs – 9.5% (EFSA & ECDC reports). We found multi-resistance in clinical isolates of *Campylobacter jejuni* at the level of 1.2% (2/162), isolates of *Campylobacter coli* at the level of 8.3% (9/109), isolates of *Campylobacter lari* – 4.5% (1/22). Multiresistance from broilers was shown by 16.7% (1/6) of *Campylobacter jejuni* isolates and 7.1% (1/14) of *Campylobacter coli* isolates. The isolate from the turkey was multiresistant to the tested antibiotics. In Argentina, 77% of *Campylobacter coli* isolates were found to be resistant to five antibiotics (Schreyer et al., 2022).

Well-known methods of long-term storage of microbial cultures include cryopreservation and lyophilization. Using low temperatures with various cryoprotectants to preserve bacteria is a traditional practice in any microbiological laboratory. Low-temperature storage ensures a high level of microbial cell viability, and cryoprotectants prevent ice crystal formation during freezing (Prakash et al., 2020). However, maintaining low-temperature conditions requires significant energy consumption, specialized refrigeration equipment, and uninterrupted operation. Lyophilization provides a high level of culture viability over several years. Research (Obukhovska et al., 2015) has shown that with the preservation of lyophilized *Campylobacter* cultures, microbial cells lose 1.0% of their viable potential annually, and after 10 to 12 years, only 44.0% of strains retain their viability. In our study, one year after lyophilization and storage at –18 ± 2 °C, the viability was 80.0%.

The Commission Implementing Decision (EU) 2020/1729 of 17 November 2020 on the monitoring and reporting of antimicrobial resistance in zoonotic and commensal bacteria requires that resistant *Campylobacter* isolates be stored at –80 °C for at least 5 years. In response, we initiated the creation of a unique, first-in-Ukraine collection of *Campylobacter* isolates, encompassing representatives from all regions and species of animals, poultry, and humans. This collection aims to study the molecular genetic and biological properties of *Campylobacter* and to serve as a repository for tracking the evolution of isolates over time.

Several conditions had to be met for creating the collection:

– prevent cross-contamination that could compromise the study of their molecular genetic properties;

– ensure that microbial cell viability remains close to the original level for as long as possible.

To address the challenge of long-term preservation of *Campylobacter* isolates, we evaluated existing methods such as freeze-drying and freezing at –70 ± 5 °C. Given the requirement for continuous, uninterrupted electricity for low-temperature freezers, we decided against cryopreservation. Although some researchers have used this method for creating *Campylobacter* collections (Madden & Madden 2019; Al-Khresieh et al., 2023), our study found that lyophilized *Campylobacter* isolates stored at –18 ± 2 °C for 12 months lost only 0.15% of their viable microbial cells compared to the initial level. Therefore, we concluded that lyophilization is the most suitable method for long-term storage of *Campylobacter* isolates in our study.

Our results showed that *Campylobacter coli* dominates the Ukrainian poultry industry. Antimicrobial drugs, especially groups of macrolides, fluoroquinolones and tetracyclines, are widely used in poultry farming, which contributes to the emergence of resistant commensals and zoonoses. The state policy of Ukraine on the use of antimicrobials in raising livestock for slaughter includes state monitoring of residues of veterinary drugs and contaminants in poultry, pig and cattle meat. According to Order No. 97 for implementing Delegated Regulation of the Commission (EU) No. 2022/1644, supplementing Regulation (EU) 2017/625, in 2023 samples of poultry meat were tested (n = 3170), beef (n = 870) and pork (n = 1060). The material was selected from farms in 22 regions of Ukraine. Among the antibiotics are representatives of the group of fluoroquinolones, in particular, ciprofloxacin, macrolides – erythromycin and tetracycline. No positive samples were found. Monitoring of *Salmonella* spp. in the poultry industry, according to the "Instructions for the Prevention and Elimination of Poultry Salmonellosis" by Order No. 310, provides for the examination of poultry droppings for the content of macrolide, fluoroquinolones, and tetracycline antibiotics. In this way, there is a double-controlled use of antimicrobial drugs in poultry farming to prevent the emergence of antibiotic resistance.

Such a study was conducted in Ukraine for the first time. The obtained results confirm the circulation of antibiotic-resistant isolates of *Campylobacter* spp. and in samples from children for campylobacteriosis, and in natural sources of infection. The dominance in poultry farming of *Campylobacter coli* isolates, which acquire resistance to antimicrobial drugs of, was revealed. In our study, we found persistent resistance of *Campylobacter* spp. to ciprofloxacin and tetracycline. In general, data on the resistance of isolates of *Campylobacter* spp. in Ukraine coincide with global trends.

Conclusion

Our study highlights the important issue of antimicrobial resistance (AMR) in *Campylobacter* species isolated in Ukraine, focusing on resistance to fluoroquinolones and macrolides, two critical classes of antibiotics. Studies have revealed a high level of resistance of *Campylobacter* isolates both from clinical settings (children with acute intestinal infections) and from agricultural sources (animals and poultry). Of particular concern is the high resistance to ciprofloxacin and tetracycline, as well as the presence of multidrug resistance (MDR) in a subset of isolates.

The study highlights the role of *Campylobacter* as a commensal organism in the gut of mammals and birds, facilitating the horizontal transfer of AMR determinant genes, which enhances the spread of resistance. The results highlight the urgency of further research into the molecular mechanisms of resistance and the genetic determinants involved, as well as the need for ongoing monitoring and strategies to control AMR in both humans and veterinary medicine. Establishing a complete collection of isolates and establishing optimal storage conditions is a valuable resource for future research to address this growing public health problem in Ukraine.

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The authors declare no conflict of interest.

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