Research Article

Prevalence and Antimicrobial Resistance of Campylobacter Isolated from Animals and Poultry in Ukraine

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Abstract | Campylobacteriosis is the most frequent etiological form in a structure of acute intestinal infections, species of *Campylobacter* spp. which are the main food pathogens. In Ukraine, the distribution of emergency campylobacter has not been researched. This article describes conditions for active monitoring of *Campylobacter* spp. antimicrobial resistance, selection and research of 2,120 samples of cecal contents from cattle, pigs, and poultry. The samples were analyzed by a microbiological method for the detection of *Campylobacter* according to the scheme of accumulation, isolation, isolation and identification according to DSTU ISO 10272-1:2007. As a result, 33 isolates of *Campylobacter* spp. were isolated, which is 1.6% of the total number of the researched samples. The most common phenotypes of antimicrobial resistance of the selected isolates were: Cip / Tet / Ery - 14 isolates from poultry, which accounted for 42.42%. Tet / Ery - 4 isolates from poultry (12.12%), Cip / Tet - 1 isolate from poultry (3.03%), Cip / Ery - 1 isolate from pigs (3.03%). The presence multidrug resistant (MDR) *Campylobacter* isolates obtained from broilers indicates dangerous uncontrolled use of antibiotics in poultry farming. The article highlights the potential health hazard to consumers posed by the presence of *Campylobacter jejuni* in industrial poultry and poultry products, as well as its resistance to various antibiotics. The study also reports a high rate of detection of resistant *Campylobacter* spp. among agricultural animals and the population in Ukraine, necessitating the introduction of an effective strategy for systematic monitoring of campylobacteriosis. Research results showed a high rate of detection of resistant *Campylobacter* spp. in Ukraine, circulating both among agricultural animals and among the population. Such monitoring can help regulate and contain the occurrence of food zoonoses and mitigate their impact on consumers of agricultural products.

Keywords | Active monitoring, Multidrug resistant (MDR) *Campylobacter* in poultry farming.

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INTRODUCTION

The *Campylobacters* are one of the main causes of acute intestinal infections of bacterial etiology with oral transmission and pose a significant potential danger to public health (WHO, 2020). *Campylobacter* is a commensal bacterium that inhabits the gastrointestinal tract of farm animals, domestic and wild poultry, and can be transmitted

to humans through the consumption of contaminated food or contact with infected animals (Facciolà et al., 2017). The main food pathogens includes: species *Campylobacter jejuni, Campylobacter coli* and *Campylobacter lari.* (Hansson et al., 2018). *Campylobacter jejuni* and *Campylobacter coli* are quite relevant pathogens of food-borne campylobacteriosis among the population of the European Union (EFSA 2019, 2020, 2021) and the United States (FoodNet 2019, 2020, 2021) compared to others representatives of the genus. Poultry, especially broiler chickens, are considered the main natural source of *Campylobacter jejuni* and the main object of monitoring (Sibanda et al., 2018; Igwaran et al., 2019). According to the report of the European Union about zoonoses in One Health section, the percentage of broilers affected by representatives of the *Campylobacter* genus in the EU countries is: Romania - 85.85%, Greece - 76.37%, Spain - 63.84%, Czech Republic - 62.11%, Germany - 49.82%, Slovakia - 47.54%, Austria - 46.90%, Latvia - 33.33%, Switzerland - 30.57%, Estonia - 11.76% (EFSA, 2021). Mass consumption of poultry meat and meat products has been associated with a high incidence of campylobacteriosis cases. Among the various sources of *Campylobacter* infection, poultry meat and meat products are considered a major source of human infections, accounting for 50-70% of all reported cases of campylobacteriosis (Facciolà et al., 2017; Chlebicz et al., 2018). Experts justify this dangerous statistic with the rapid development of poultry farming and the growing of poultry meat production, which is an ideal distic product (low calorie and high protein content), an important source of vitamins and minerals, affordable, quick to prepare and not inferior in terms of taste qualities of any other type of meat. A third of the population prefers exactly him (Thames et al., 2020; Hakeem et al., 2021). In most cases, contamination of poultry meat and meat products has an exogenous origin. During the slaughter process, there is a risk of contamination of meat with the contents of the gastrointestinal tract, which can contain harmful bacteria such as *Campylobacter*. If proper hygiene and sanitation measures are not followed during processing, handling, and preparation of meat, the risk of contamination and subsequent food poisoning increases. This is why it is important to follow proper food safety practices and regulations to reduce the risk of foodborne illness from contaminated meat (Zhang et al., 2018; Hakeem et al., 2021).

According to the Centers for Disease Control and Prevention (CDC), Campylobacter infections are estimated to cause 1.5 million cases in the United States every year. The statement is also correct in mentioning the Foodborne Diseases Active Surveillance Network (FoodNet), which is a surveillance system that monitors the incidence and trends of foodborne diseases in the United States. Thus, there are about 20 reported cases of campylobacter per 100,000 in the United States over the last few years. The National Antimicrobial Resistance Monitoring System for Enteric Bacteria (NARMS) has been monitoring *Campylobacter* spp. since 1998, as a foodborne pathogen. Since the beginning of 2007, *Campylobacter spp.* has been included in the list of zoonotic pathogens monitored for antibacterial drug resistance in European Union countries. Statistics of confirmed cases of human campylobacteriosis in EU countries for 2020 amounted to 120,946 cases (40.3 per 100,000 population), for 2021 - 127,840 cases (41.1 per 100,000 population) (EFSA, 2021).

The distribution of *Campylobacter* in Ukraine has been explored to some extent, as evidenced by the study mentioned earlier. However, further research is needed to fully understand the distribution and prevalence of the pathogen in Ukraine, particularly with regards to potential carriers and sources of infection.

According to the Public Health Center of the Ministry of Health of Ukraine, the incidence of acute intestinal infections and food poisoning with unknown etiology per 100,000 population increased from 46.8 in 2020 to 57.08 in 2021. Confirmed cases of campylobacteriosis in 2020 (0.37 per 100,000 population), in 2021 (0.45 per 100,000 population), which of 44% are acute intestinal infections of patients pediatric infectious hospitals of the city Dnipro. The most vulnerable in terms of susceptibility to campylobacteriosis are children up to one year - 8.4%, from 1 to 3 years - 37%, from 4 to 10 years - 43.4%, and over 10 years - 11.2%. Obviously, this is consistent with the data of foreign experts, in developing countries, campylobacteriosis is mainly a problem for children compared to the adult population (Carron et al., 2018; Natsos et al., 2018).

In Ukraine, *Campylobacter* is not obligated in the list of "Microbiological criteria for establishing food safety indicators", in contrast to classic food pathogens such as *E.coli, Listeria monocytogenes* and *Salmonella* (Kozytska et al., 2023). Therefore, providing the microbiological safety of food products with regard to *Campylobacter* remains unresolved.

The specific requirements to growth of *Campylobacters*, which are microaerophiles, capnophiles, thermophiles and grow well at an optimal water activity of 0,997, can make it difficult to detect them in laboratory research (Hansson et al., 2018; Igwaran et al., 2019; Myintzaw et al., 2021). The difficulties of cultivating and isolating *Campylobacter*, the high cost of laboratory diagnostics and the absence of obligatory studies explains the absence of work reports of State laboratories of veterinary medicine of the State Service of Ukraine on Food Safety and Consumers Protection (SSUFSCP) of positive cases of campylobacteriosis studies of an-

imal and poultry for the period from 2009 to 2021.

Therefore, the purpose of our research was to: 1) monitor the spread of *Campylobacter spp.* among livestock and poultry in Ukraine; 2) evaluate the resistance of isolated *Campylobacter spp.* strains to antimicrobial drugs; and 3) conduct a comparative analysis of the monitoring results in terms of geography and time.

MATERIALS AND METHODS

Monitoring design and sampling

The program "State monitoring of antimicrobial resistance in veterinary medicine" was carried out for the first time and was financed from the State Budget of Ukraine. In order to harmonize monitoring and obtain objective data, the researchers were guided by Commission Implementing Decision (EU) 2020/1729 "…on monitoring and reporting on antimicrobial resistance in zoonotic and commensal bacteria". The 216 farms participated in the research from all regions of Ukraine, except the temporarily occupied territories of Donetsk, Luhansk regions and the annexed Crimea.

In our investigation, 23% of the farms were poultry farms, 44% were pig farms, and 33% were dairy and meat cattle farms. All farms had industrial technology for keeping and growing agricultural animals and poultry, with meat production exceeding 10,000 tons per year. Some of the materials were collected from pigs and cattle kept in private peasant farms, which were purchased by processing enterprises for slaughter.

Specialists of the SSUFSCP in the regions, in accordance with order № 514 "On the approval of the State Monitoring Plan for Antimicrobial Resistance in Veterinary Medicine for 2021", organized the selection, accounting, labeling, storage and transportation of samples to the reference laboratory - the State Scientific Research Institute of Laboratory Diagnostics and Veterinary and Sanitary Expertise (SSRILDVSE, Kyiv).

Samples were collected using a random sampling scheme directly during slaughter at 176 slaughterhouses, including those at meat processing enterprises, meat processing plants, and poultry farms. The selected slaughterhouses accounted for approximately 60% of the total slaughter of domestic animals and poultry in Ukraine. Samples were also collected at other slaughterhouses on a random basis. The animals and poultry subject to selection were of slaughter age. Poultry was slaughtered at the age from 27 to 54 days, as well as 3, 5 and 7 months, pigs - from 5 to 8 and 12 months of age. The age of cattle is up to 2.5 years, contrary to the decision of the Commission (EU) 2020/1729 - up to one year of age. 2120 samples of cecal contents from cattle, pigs and poultry were selected for research, of which: 848 from poultry (quail - 12, ducks - 2, turkeys - 88, broiler chickens - 746), 581 samples from cattle and 691 samples from pigs. The total coverage of the study of the livestock of animals and poultry available in Ukraine was 0.07% of those purchased by processing enterprises in the IV quarter of 2021 (Ukrstat, 2021). The samples were transported to the laboratory in thermal boxes with accumulators of artificial cold to protect them from exposure to light, high temperature or freezing, atmospheric oxygen and drying.

Isolation of *Campylobacter* and their phenotypic characteristics

Campylobacter is sensitive to environmental conditions, therefore cultures on nutrient media were carried out on the day of receipt of the material. Samples that could not be processed on the same day were stored at a temperature of +4°C until the next working day. Before sowing, they were kept at room temperature to avoid temperature shock (Terrestrial Manual, 2018).

The selected samples were examined in accordance of DSTU ISO 10272-1 :2007 Microbiology of food and animal feeding stuffs - Horizontal method for detection and enumeration of *Campylobacter spp.* - Part 1: Detection method (ISO 10272-1:2006, IDT) and OIE Terrestrial Manual 2018 / Chapter 3.10.4. – Infection with *Campylobacter jejuni* and *Campylobacter coli.* All media and selective additives to them were used manufactured by HiMedia, Mumbai, Maharashtra, India.

The test material was sown in the ratio of 1:9 in the accumulation medium - Bolton Broth Base (M1592) with the addition of 5% Horse Blood Lysed (HB037) (TCS Biosciences Ltd, United Kingdom) and Bolton Selective Supplement (FD231) was incubated in a microaerobic atmosphere firstly at a temperature of 37°C for 4 hours and then at a temperature of 41.5°C for 44 hours. Reseed were made on Blood Free Campylobacter Selectivity Agar Base - mCCD agar (M887) with the addition of Campylobacter Supplement V (BFCSA) (FD067) and a Preston Agar Base (M939) with the addition of Campylobacter Selective Supplement IV (Preston) (FD042) and 25 cm^3 Horse Blood Lysed (HB037) (TCS Biosciences Ltd, United Kingdom). Incubated by a temperature of 41.5°C for 44 hours in microaerobic conditions. 5 typical colonies were selected from each selective medium and sown with a stroke on plates with Columbia Blood Agar Base (M144), incubated by a temperature of 41.5°C for 48 hours. After viewing cultures, one colony was dispersed from Columbia Blood Agar Base (M144) in 1 cm^3 of Brucella Broth Base (M348) and tested for motility in a dark field. Staining of smears according to Gram and microscopy were car-

ried out. Oxidase production was detected using Oxidase Discs MD018) (HiMedia, India) and catalase activity in relation to a 3% solution hydrogen peroxide. Microaerobic incubation conditions were created with the help of gas-generating packages Anaerogas Pack 3.5L (LE002A-SNO) (HiMedia, India).

Antibiotic sensitivity test

Sensitivity testing of isolates of *Campylobacter spp.* to antibacterial drugs, carried out by the disk diffusion method according to EUCAST Version 9.0 (January 2021). To prepare the inoculum, typical colonies of a 40-hour culture on Columbia Blood Agar Base (M144) were selected suspended in NaCI solution (0.9%) to a density of 0.5 on the McFarland standard scale (R092) (HiMedia Laboratories, India). After applying the inoculum to the surface of Mueller Hinton Agar (M173) with the addition of 5% Horse Blood Lysed (HB037) (TCS Biosciences Ltd, United Kingdom), triturated it, evenly hatching in three directions, turning the Petri dish. Then discs were placed with the antibiotics Ciprofloxacin 5 μg (CIP) (SD060), Tetracycline 30 μg applied to them (TE) (SD037), Erythromycin 15 μg (E) (SD013) (HiMedia Laboratories, India) and cultivated under microaerobic conditions at 41.5°C for 48 hours. After incubation, using a special ruler by (HiMedia Laboratories, India), the diameters of the growth inhibition zones of the studied *Campylobacter* isolates were measured to the nearest millimeter, and the results were interpreted in accordance with the recommendations EUCAST version 11.0, 2021-01-01 (https://www.eucast.org/ast_of_bacteria).

Statistical analysis

Digital research results are presented in accordance with the requirements for the International System of Units and statistically processed using the Microsoft Excel computer program. The probability of the difference between the groups by different indicators was estimated using the Student t-criterion when comparing averages (the difference was considered likely by $p<0.01$) (Sergeant ESG 2019).

RESULTS AND DISCUSSION

Bacteriological studies

On the first stage, a total of 2120 samples of cecal contents were collected from cattle (n=581), pigs (n=691), and poultry (n=848) in 216 farms. Of the 581 cattle samples, 463 were collected from animals on industrial farms, and 118 were from private peasant farms. The number of cattle on farms of slaughter age (up to 2.5 years) was 287,000 heads, and the 463 samples represent 0.16% of the total examined population.

For pigs, 691 samples were collected from 96 farms, including 676 samples from animals on industrial farms and 15 from private peasant farms. The number of pigs on farms of slaughter age was 162,000 heads, and the 676 samples represent 0.42% of the total examined population.

For poultry, 848 samples were collected from 49 farms. All samples were analyzed using a microbiological method for the detection of Campylobacter, following the scheme of accumulation, isolation, and identification according to DSTU ISO 10272-1:2007. In the course of monitoring studies, 33 isolates of *Campylobacter spp.* were isolated, of which 1.6% of the total number of examined samples. The biggest number of *Campylobacter spp.* in the material collected were isolated from poultry (broilers) - 26 isolates, from cattle - 5 isolates and 2 isolates from pigs.

We used the enrichment method to increase the level of Campylobacter isolation in the contents of the caecum of animals (Terrestrial Manual, 2018). Reception to application for isolation of *Campylobacter spp.* the method of accumulation in Bolton Broth Base contributed to the growing of the microflora that was tried to be detected. The intensive growth of concomitant (secondary) microflora complicated the work, which was tried to get rid of by using a selective additive containing antimicrobial drugs and creating conditions for incubating crops at 41.5°C.

The selective additive (FD231) includes Cefoperazone, which is designed to inhibit the growth of *Enterobacteriaceae* microorganisms, but, according to recent data, *Escherichia coli* has acquired persistent resistance to β-lactams, in particular to Cefoperazone (52.9%) (Kadry et al., 2022).

However, it was inpossible to completely overcome the contamination of crops with species of normal intestinal microbiota, which reduced the effectiveness of studies on the detection of *Campylobacter* spp. In our opinion, one of the reasons for the decrease in the growth of *Campylobacter* spp. could be the presence of commensal bacteria in the samples that produce bacteriocins. This property is considered a probiotic feature that reduces the load of *Campylobacter* spp. in the body *Enterococcus spp.* (Lin 2009) is one of the bacteria that exhibit this property, and 10.7% of the isolates were found during monitoring. However, the enrichment method used in the detection process may promote the growth of secondary microflora, which could make it difficult to detect *Campylobacter* spp. In the EU, specialists resorted to isolating *Campylobacter spp.* by direct plating, neglecting the enrichment method, which falsely showed negatives results (Jorgensen et al., 2022).

After 44 hours of cultivation on mCCD agar, the growing of small, flat, wet, grayish colonies with a metallic sheen was discovered (Fig. 1). On Preston Agar Base - cultures

formed wet, gray, flat growth with a tendency to coalesce. On Columbia Blood Agar Base - homogeneous, small, dewy, transparent colonies with a bluish-gray tint, without a zone of hemolysis (Fig. 2).

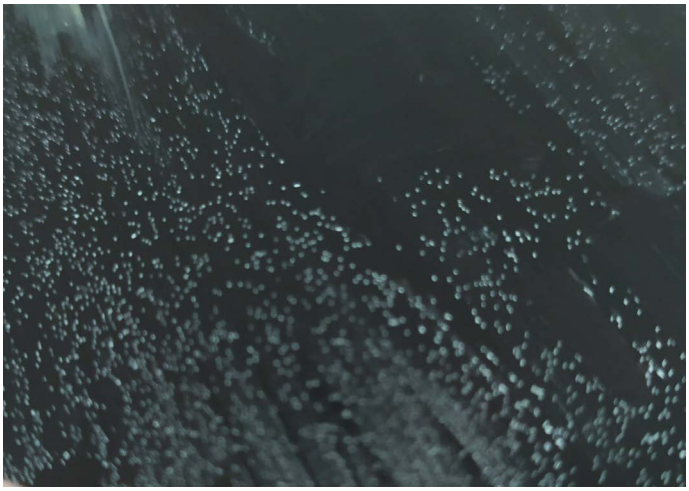

Figure 1: Growth of a 44-hour culture Campylobacter on mCCD agar.

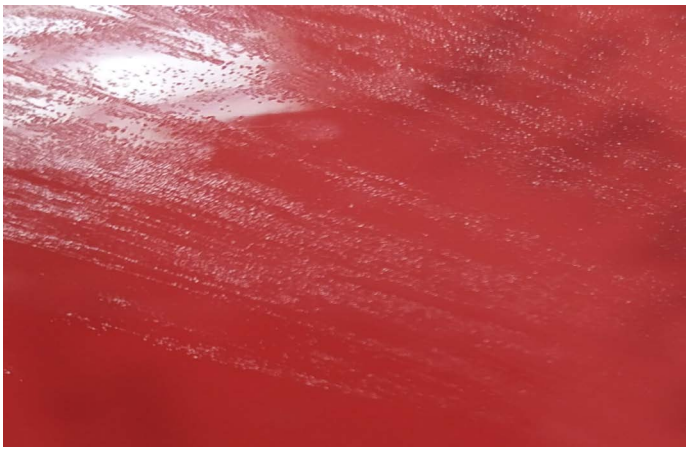

Figure 2: Growth of Campylobacter on Columbia Blood Agar Base.

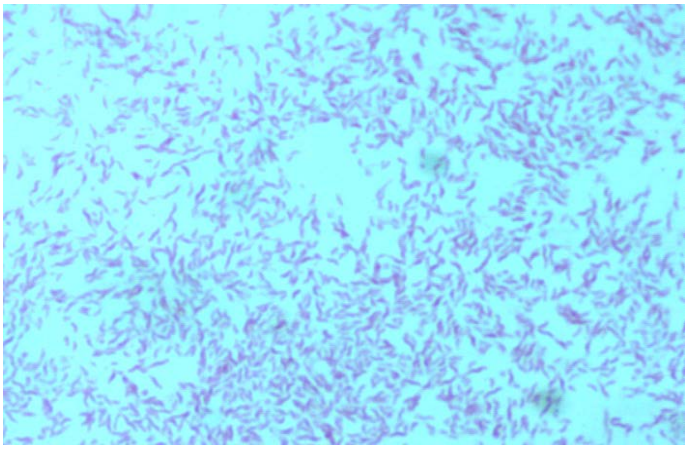

Figure 3: Accumulation of *Campylobacter* bacteria in the smear. Gram stain, viewed under a Leica DM 5000 light microscope (100 immersion objective) using a Leica DFC 295 camera and Leica LAS X software

When studying the morphological and tinctorial properties of culture isolates obtained on nutrient media, polymorphic thin bent Gram-negative rods were recorded in smears. They were located in the form of a comma or a flying seagull, the letters S or V, a spiral with one or several curls (Fig. 3). The motility of microorganisms during phase-contrast microscopy of preparations was characterized by rapid spiral-like and «screw-like» changing in the position of microbial cells.

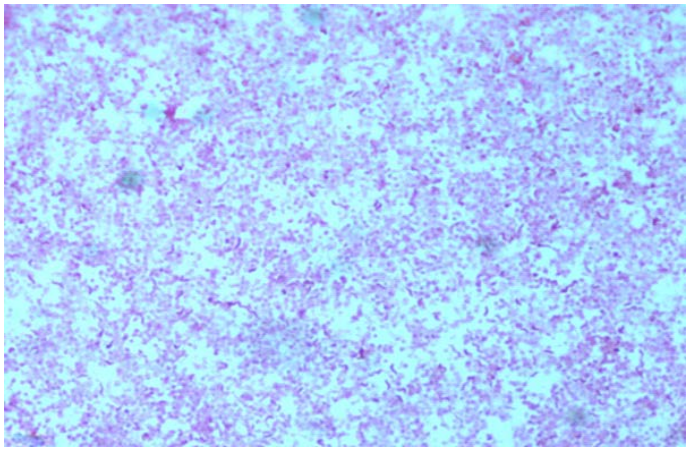

Figure 4: Transition of the spiral form of Campylobacter to the cocci form.
a - spiral form; ***b*** - the cocci form of *Campylobacter*. Gram stain, viewed under a Leica DM 5000 light microscope (100 immersion objective) using a Leica DFC 295 camera and Leica LAS X software

These figures demonstrate the cultural (colonies on selective nutrient mediums are small, flat, moist, dewy from transparent with a bluish-gray tint to grayish color with a metallic luster and a tendency to grow) and morphological (polymorphic thin bent Gram-negative rods in the form of commas or seagulls, which flies, letters S or V, spirals with one or several curls) properties of selected isolates, typical for *Campylobacter* spp., which is consistent with other authors (Vandamme et al., 2015; Natsos et al., 2019; Abdul-Aziz et al., 2022).

Work on reseeding, making smears, and testing selected isolates of *Campylobacter spp.* was carried out promptly. An uncontrolled increase in the concentration of oxygen in the gas environment of bacterial cultures and their long-term storage on nutrient media caused *Campylobacter* to lose its spiral shape and acquire a coccoid form, which is a viable but not cultivable state (VBNC) (Fig. 4) (Ikeda et al., 2012; Ammar et al., 2019; Frirdich et al., 2019).

By studying the biochemical properties of the isolated strains, it was established that all of them tested positive for catalase, indicating their pathogenicity. *Campylobacter* did not ferment carbohydrates, produce indole, liquefy gelatin, form hydrogen sulfide, cause hemolysis on blood agar,

or produce oxidase.

For informational purposes, we identified 10 isolates of Campylobacter spp. from broiler chickens in different regions of Ukraine using MALDI-TOF mass spectrometry on a VITEK MS device. It was found that all 10 isolates belonged to the species *Campylobacter jejuni*. These results suggest that *Campylobacter jejuni* is the most common species of the *Campylobacter* genus, and that broiler chickens are its main reservoirs (Sibanda et al., 2018; Igwaran et al., 2019).

Distribution of *Campylobacter spp.* in Ukraine

The results of determining the geographical distribution of *Campylobacter spp*. in agricultural animals and poultry from farms using industrial and traditional keeping technologies, which were carried out in the Dnipropetrovsk, Lviv, Cherkasy, Chernihiv, Kherson, and Khmelnytsky regions, are shown in Figure 5.

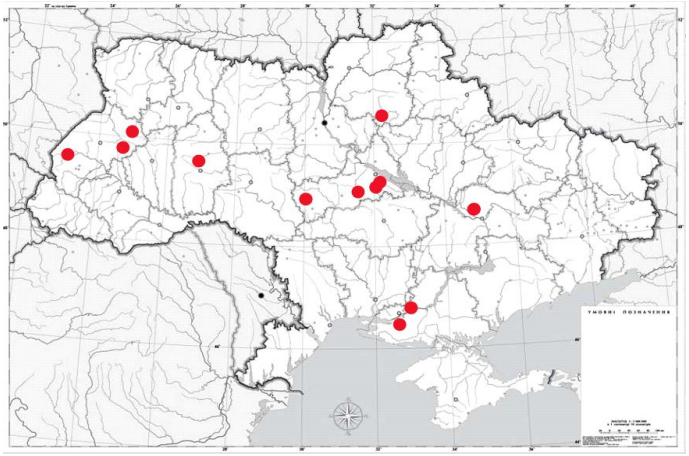

Figure 5: Regions of Ukraine with different levels of campylobacteriosis in animals and poultry farms.

More detailed analyses showed that poultry farms in the Lviv, Cherkasy, Chernihiv, and Khmelnytskyi regions, where positive samples for Campylobacter spp. were detected, were using closed-loop technology. The poultry houses were equipped with an automated system for microclimate control, drinking, and feeding, and the birds were kept on the floor with deep litter. The farms used their own modern slaughterhouses with a wide range of operations, including butchering, automated cutting of poultry, packaging, cooling, and freezing. The farms imported hatching eggs and one day-old chicks from EU countries periodically to update the parent herd and preserve the breed's characteristics. However, while salmonellosis was being monitored and controlled, campylobacteriosis was not being researched as a zoonotic disease.

In the Kherson and Cherkasy regions, pig farms were engaged in breeding pigs with a closed production cycle, obtaining piglets from their own mother herd, rearing, and fattening animals before selling them to meat processing enterprises. The premises met the requirements of the sanitary and hygiene regime, and pig fattening was based on the use of feed of own production.

In the Cherkasy region, a farm specialized in milk production and dairy cattle breeding. The animals were kept untethered and distributed according to the age and physiological principle. Livestock premises were equipped with a supply-exhaust ventilation system, mechanical manure removal, and automatic watering. Fodder for older animals was distributed on the feed table, and for young animals in the feeder. The farm's feed supply was based on feed of own production and had a multi-component diet.

Modernization of the technological process and intensive production can create a microbial load on the animal body, the composition of which directly depends on the parameters of the microclimate of the premises, hygiene of housing, density of planting, quality of litter and feed, balanced diet, species of animals, and even their health. As a result, there is an imbalance of the body's microbiota, which suppresses immunity and increases the frequency of diseases (Chmielowiec-Korzeniowska et al., 2020; Soliman et al., 2020).

Four isolates of Campylobacter spp were found among cattle in 13 regions of Ukraine on private peasant farms. Two isolates were found in the Lviv region, and one each in the Dnipropetrovsk and Kherson regions, which corresponds to 3.4% of the total isolates. This indicates that Campylobacter is widely distributed in nature, and its main sources are environmental pollution, such as pastures and water bodies, which contribute to cross-contamination.

Thus, the analysis of Ukraine farms allows us to conclude that systematic monitoring and surveillance of zoonotic and commensal bacteria are necessary to improve the biosecurity of farms. This confirms that public health is closely related to animal husbandry as humans are consumers of agricultural products, which increases the risk of infection with nosocomial bacterial infections, such as campylobacteriosis.

Detection of resistance to antimicrobial drugs in *Campylobacter spp.*

All the selected isolates were tested for their resistance to antimicrobial drugs, and the results showed that some Campylobacter spp. isolates were resistant to the tested antibiotics, On next stage was collected host information on each Campylobacter isolate, its resistance and the percentage of resistant isolates selected by animal species and the total number (Table 1).

Table 1: Resistance of selected isolates to antimicrobial drugs.

Antibiotic	**Resistant *Campylobacter* spp. / Number of isolates**							
	Broilers		Bovine animals		Pigs		Total	
	absolute	percentages	absolute	percentages	absolute	percentages	absolute	percentages
Ciprofloxacin/ 5μg	15/26	57,7/100	1/5	20/100	2/2	100/100	18/33	54,5/100
Erythromycin/ 15μg	19/26	73/100	0/5	-	1/2	50/100	20/33	60,6/100
Tetracycline/ 30μg	23/26	88,5/100	2/5	40/100	0/2	-	25/33	75,8/100

This table shows the characteristics of 33 isolates of Campylobacter spp. - 26 isolates from broilers, 5 isolates from cattle and 2 isolates from pigs. Of the 26 poultry isolates, 15 are resistant to ciprofloxacin, which is 57.7% of those isolated from birds, 19 are resistant to erythromycin, respectively 73%, and 23 are resistant to tetracycline, which is 88.5%.

Of the total number of tested isolates, 18 are resistant to ciprofloxacin, which is 54.5%, 20 are resistant to erythromycin, which is 60.6%, and 25 are resistant to tetracycline, which is 75.8% of the total number of isolated isolates.

Our study found that 33 isolates of *Campylobacter spp.* exhibited consistent resistance to tetracyclines (75.8%), macrolides (60.6%), and fluoroquinolones (54.5%).

Additionally, we found that four isolates (two from poultry and two from cattle) were fully sensitive to all the tested antimicrobial drugs, which represents 12.1% of the total isolates.

In addition, out of the tested isolates, 15 were resistant to one or two antimicrobial drugs (10 from poultry, 3 from cattle, 2 from pigs). Figure 6 displays the distribution of isolates based on animal species, showing the number of isolates that exhibit full sensitivity, resistance to 1-2 antimicrobial drugs, and multiresistance (MDR).

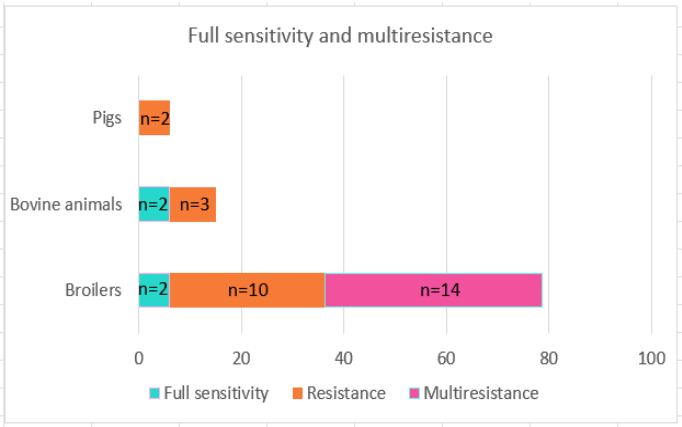

Figure 6: Full sensitivity and multiresistance of isolates of Campylobacter spp. isolated during monitoring for antimicrobial resistance.

The figure displays the number of *Campylobacter spp.* isolates from animal and their resistance to antimicrobials. Based on our investigation, we identified the following antimicrobial resistance phenotypes among the isolates: Cip/Tet/Ery - 14 isolates from poultry, which represents 33.33% of the total isolates from poultry. Four isolates from poultry (9.09%) were found to be resistant to Tet/Egu, one isolate from poultry (2.27%) was resistant to Cip/Tet, and one isolate from pigs (2.27%) was resistant to Cip/Ery. Therefore, resistance to the three classes of antimicrobials tested indicates the excessive use of antimicrobials in the poultry industry. Taking into account the findings from previous studies (Béjaoui et al., 2022; Popa et al., 2022) and our results, we conclude that broilers are the primary source of human campylobacteriosis, and the persistent AMR resistance of Campylobacter spp. is a food safety problem that poses a significant potential threat to public health. Strengthening surveillance of the foodborne pathogen Campylobacter spp. in primary production, which is a potential source of the spread of antimicrobial resistance, would curb the increasing impact of antimicrobial resistance on animals, humans, and the environment.

Analysis of resistance to antimicrobial drugs *Campylobacter spp.*

Taking into account the results obtained by us, we conducted a comparative study in the data of other researchers. According to National antimicrobial monitoring systems resistance (NARMS) of the USA and the European Union consolidated report on antimicrobial resistance in zoonotic and indicator bacteria (EFSA and ECDC) most *Campylobacter* strains indicate persistent resistance to tetracycline and ciprofloxacin. Studying of the influence of geographical localization of the persistence of *Campylobacter spp.* on the manifestation of antibiotic resistance was carried out by comparing the degree of manifestation of resistance of selected isolates in the USA, in the EU countries and in the Ukraine. The obtained data helped to find out that isolates of *Campylobacter spp.* of various origins generally have persistent resistance to the tested groups of antibiotics (fluoroquinolones, macrolides, and tetracyclines) (Fig. 7, 8, 9).

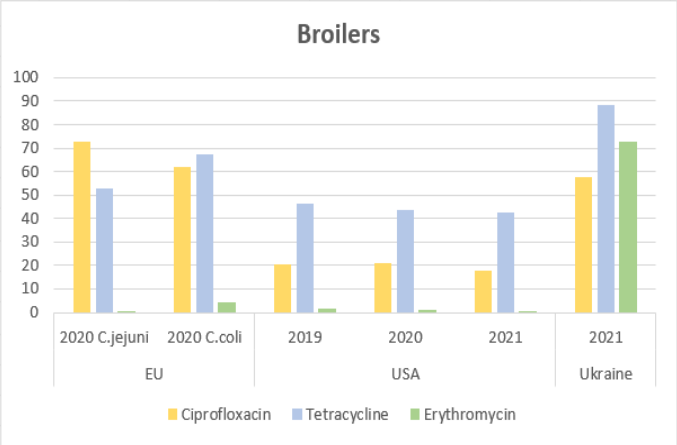

Figure 7: Comparison of the resistance of selected isolates from broilers in the EU, USA and Ukraine.

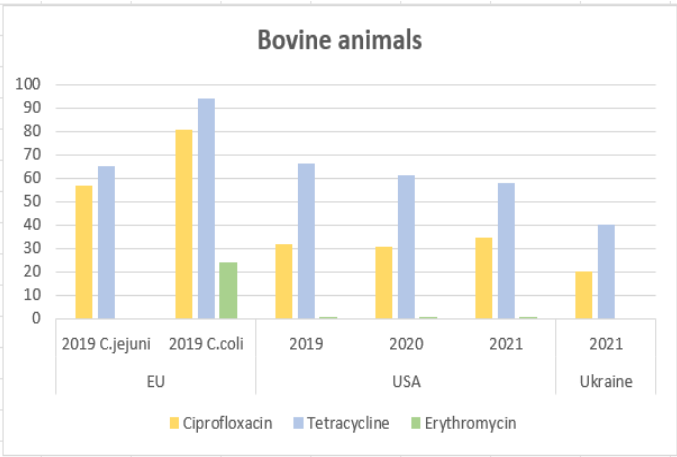

Figure 8: Comparison of the resistance of selected isolates from cattle in the EU, USA and Ukraine.

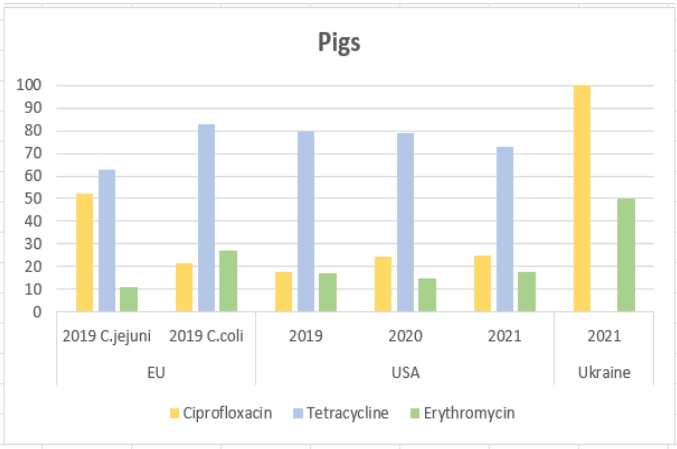

Figure 9: Comparison of the resistance of selected isolates from pigs in the EU countries, the USA and Ukraine.

These figures shows that in the USA, according to the latest data, the resistance to tetracycline of isolates from broilers is 42,5%, from cattle – 57,9%, from pigs – 72,7% in the EU tetracycline resistance of *Campylobacter jejuni* isolates from broilers – 52,7%, from cattle – 65,3%, *Campylobacter coli* isolates from pigs – 82,8%. In Ukraine, the resistance of selected isolates to tetracycline from broilers is 88,5%, cattle - 40%, pigs - there is no resistance.

In the USA, broilers are resistant to ciprofloxacin – 17,8%, cattle – 34,3%, pigs – 25,1%. In the EU: broilers – 72,8%, cattle – 56,8%, pigs – 52,4%. In Ukraine: broilers – 57,7%, cattle - 20%, pigs - 100%.

The main difference between the monitoring results was that the isolates of Campylobacter spp., isolated in the USA during studies of resistance to erythromycin, had low resistance: broilers - at the level of 0,8%, cattle – 0,4%, pigs – 17,6%. Similar data were found in EU countries: broilers – 0,8%, cattle - sensitive, pigs – 27,1%. In Ukraine, the isolates isolated from chickens had a high resistance to erythromycin - 73%, from cattle - there is no resistance, from pigs - 50%.

The combined resistance of Cip / Ery, which is considered critically important for the treatment of campylobacteriosis, in Ukraine was: from chickens and cattle - 0%, from pigs - 50%. At the same time, in the EU, the resistance of *Campylobacter jejuni* isolates from broilers is 0,7%, from calves - 0%, and *Campylobacter coli* isolates from pigs – 8,0%. In the USA, the combined resistance of Cip / Ery isolates from broilers is 0%, from cattle – 0,3%, from pigs – 7,6%. Cip / Ery / Tet multiresistance of European *Campylobacter jejuni* isolates from broilers was 0,8%, cattle – 1,4%, *Campylobacter coli* from pigs – 7,8%. In the USA, the complete resistance of isolates from broilers is 0%, from cattle – 0,3%, from pigs – 7,6%. In Ukraine, multiresistance is noted only in isolated isolates from poultry, which is 42,42%.

Analysis shows that Campylobacter acquires resistance to clinically important antibiotics. Scientists from many countries have investigated and recognized that the resistance of Campylobacter to drugs of first choice for the treatment of human campylobacteriosis has recently increased. In the US, it caused about 310,000 cases of incurable infections, resulting in 0.01% of deaths annually (Yang et al., 2019). In EU countries, the resistance of Campylobacter jejuni to ciprofloxacin has reached a critical level, as a result, this antibiotic can no longer be used for the treatment of human campylobacteriosis (EFSA and ECDC, 2023).

In Ukraine, studies of Campylobacter resistance to antimicrobial drugs are limited and not studied due to the high cost and optionality of the studies. Therefore, the conducted «State monitoring of antimicrobial resistance in veterinary medicine» helped us identify Campylobacter spp. and determine antimicrobial resistance of isolates.

We also conducted an analysis of antimicrobial resistance

Table 2: Antimicrobial resistance of *Campylobacter spp*. selected for acute intestinal infections in children over the course of three years.

Antibiotic	Resistant *Campylobacter* spp. / Number of isolates							
	2020		2021		2022		Total	
	absolute	percentages	absolute	percentages	absolute	percentages	absolute	percentages
Ciprofloxacin/ 5μg	84/98	85,7/100	74/84	88,1/100	53/59	89,8/100	211/241	87,6/100
Erythromycin/ 15μg	2/98	2,0/100	9/84	10,7/100	3/59	5,1/100	14/241	5,8/100
Tetracycline/ 30μg	32/98	32,7/100	24/84	28,6/100	29/59	49/100	85/241	35,3/100

to three groups of antibiotics of isolates of Campylobacter spp., isolated from biological material from children with acute intestinal infections in the city of Dnipro, had persistent resistance to fluoroquinolones – 87.6%, tetracyclines – 35.3%, macrolides – 5.8%. (Table 2).

The table shows the trend of increasing resistance to ciprofloxacin for the period 2020 - 2022, which indicates a problem in the use of fluoroquinolones for the treatment of gram-negative infections of the gastrointestinal tract. Therefore, the conclusions of European experts agree with the results of Ukrainian isolates.

After analyzing the results of monitoring the antimicrobial resistance of Campylobacter isolated in the USA, EU and Ukraine, as well as statistical information on the number of resistant isolates from animals during slaughter, cases of food poisoning among the population and pediatric patients (for acute intestinal infections), it can be assumed that resistance of *Campylobacter spp.* to antimicrobial drugs is growing into a global problem. It is a well-known fact that in the treatment of infectious diseases, the formation of resistance leads to partial or complete «sterilization» of the organism. As a result, the functioning of autochthonous microflora is destabilized with the future disruption of the general metabolism, trophic processes and immunocorrective functions of the patient's organism (Andrew Chetley, 1986).

Antibiotic-resistant strains of *Campylobacter* spread during to the irrational and uncontrolled use of antibiotics (stimulation of growth, prevention of infectious diseases), intensification of anthropogenic influence (environmental pollution due to economic activity), penetration into the food chain due to contamination of raw materials and products of animal origin (Davies et al., 2010; Santos-Ferreira et al., 2022).

WHO identifies antimicrobial drugs of the fluoroquinolone groups (ciprofloxacin, norfloxacin, levofloxacin, moxifloxacin, enrofloxacin) and macrolides (erythromycin, clarithromycin, azithromycin, spiramycin, tylosin) as priority for medicine and the most effective in the treatment of bacterial infections. Macrolides are often used to prevent (metaphylaxis) diseases, as well as to stimulate growth in animals. Fluoroquinolones and tetracyclines (doxycycline, tigecycline, metacycline, tetracycline) are indispensable in the treatment of human campylobacteriosis (Roth et al., 2019; WHO, 2021).

In countries with mass production of livestock products, the use of macrolides, polymyxins, fluoroquinolones (except the USA) and cephalosporins (except EU) is approved (Roth et al., 2019). Some countries are showing positive trends in curbing the use of antimicrobials in poultry farming. For example, Denmark has restricted the use of carbapenems, fluoroquinolones and colistin. In Sweden, only isolated outbreaks of the use of antibiotics in production are known (Tedersoo et al., 2022)

CONCLUSION

The research analyzed 216 farms in Ukraine, covering all regions except for temporarily occupied territories of Donetsk, Luhansk regions, and Crimea. A total of 2,120 samples of cecal contents from cattle, pigs, and poultry were collected, out of which 33 isolates of Campylobacter spp. were identified, accounting for 1.6% of the total samples. The geographical distribution analysis revealed Campylobacter spp. detection in advanced poultry farms in six regions. The study identified the need for an assessment of the risk of spreading Campylobacter spp. and the establishment of ways to prevent zoonoses transmission.

Improved methods of bacteriological diagnosis enabled the researchers to understand the true scale of Campylobacteriosis persistence in Ukraine. The formation of multi-resistant isolates of Campylobacter spp. was identified as one of the risks associated with Campylobacter habitats, particularly in poultry farming. Of note, 42.42% of multi-resistant isolates were detected in broilers, posing a potential health hazard to consumers, as poultry and poultry products are the primary natural sources of Campylobacter jejuni.

The study established a direct quantitative relationship between cases of Campylobacter contamination in livestock products and outbreaks of acute intestinal infections in

children. We emphasize the importance of implementing a unified state system of surveillance and control of Campylobacter spp. based on the One Health approach, strengthening the supervision of antibiotics use in veterinary and humane medicine, and timely updating the treatment protocols for these pathologies.

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CONFLICT OF INTEREST

Authors do not have any conflict of interests.

NOVELTY STATEMENT

For the first time in Ukraine, an analysis was conducted to investigate the distribution of Campylobacter spp. among animals, birds, and humans. The results of active monitoring by analyzing 2120 caecal samples of cattle, pigs, and poultry revealed that 33 isolates of Campylobacter spp. were identified, which accounts for 1.6% of the total number of examined samples. The article also emphasizes the proven multidrug resistance (MDR) of Campylobacter isolates, particularly among broilers, which indicates the dangerous uncontrolled use of antibiotics in poultry farming. Furthermore, it highlights the high frequency of detection of resistant Campylobacter spp. among farm animals and the population of Ukraine.

AUTHORS' CONTRIBUTION

All authors contributed equally to the manuscript.

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