Regulatory Mechanisms in Biosystems

ISSN 2519-8521 (Print)
ISSN 2520-2588 (Online)
Regul. Mech. Biosyst.

Prevalence of chlorquinolone- and cephalosporin-resistant *Escherichia coli* in fish and fish products in Ukraine

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Article info

Received 25.03.2025
Received in revised form 29.04.2025
Accepted 19.05.2025

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Musiiets, I., Rublenko, I., Chechet, O., Horbatiuk, O., Pishchanskyi, O., Ukhovskyi, V., Rublenko, S., & Zhovnir, O. (2025). Prevalence of chlorquinolone- and cephalosporin-resistant Escherichia coli in fish and fish products in Ukraine. Regulatory Mechanisms in Biosystems, 16(2), e25073. doi:10.15421/0225073

The article is related to food safety within the framework of the One Health concept. The research results presented in the article were aimed at showing the level of prevalence of fluoroquinolone-resistant *Escherichia coli* strains and strains with acquired resistance to beta-lactams and carbapenems in fish and fish products of fish processing enterprises in Ukraine. 45 strains of *E. coli* strains were isolated from samples of common perch (*Perca fluviatilis*), mackerel (*Scomber)*, silver carp (*Hypophthalmichthys molitrix*), marble trout (*Salmo marmoratus*), gilthead bream (*Sparus aurata*), crucian carp (*Carassius carassius*), common carp (*Cyprinus carpio*), herring (*Clupea sp.*), Atlantic horse mackerel (*Trachurus trachurus*) and fish products – samples of caviar, including salmon, blue mussel (*Mytilus edulis*) in brine, sauce, oil, smoked Caspian trout (*Salmo caspius*) spines. The resistance to the representatives of clinically important fluoroquinolones of the second (ofloxacin, norfloxacin), third (levofloxacin) and fourth (moxifloxacin) generations was studied by the phenotypic method. Resistance to fluoroquinolones was inherent in 14 (31.1% of identified) *E. coli* strains. The probable production of carbapenemase (OXA-48 and OXA-48-like enzymes) by *E. coli* strains was determined using discs with meropenem (10 μg) and was suspected in 6 (13.3%) of the tested *E. coli*. The production of ESBL-enzymes was confirmed by two phenotypic methods of combined and double discs in 5 (11.1%) *E. coli* strains. The production of AmpC-enzymes was confirmed by the phenotypic method for resistance to cefotaxime (30 μg) in 6 (13.3%) *E. coli* strains. The results of the study provide a justification for inclusion in the state monitoring to detect contamination of raw materials and products of fish processing enterprises in Ukraine with bacterial contaminants. Such monitoring will help to strengthen quality control and bio-safety of fishery raw materials and products as one of the links in the food chain in the One Health system.

Keywords: *Hypophthalmichtys molitrix*; *Carasius carasius*; *Salmo marmoratus*; *Mytilus edulis*; fluoroquinolones; cephalosporins; ESBL enzymes; AmpC enzymes; OXA-48; OXA-48-like enzymes.

Introduction

At the current stage of Ukraine's integration into the European Union (EU) and the international community, the main priority areas of the global One Health strategy are to preserve the health of humans, animals and poultry in the context of high-quality and safe production. Such requirements affect life, performance, disease resistance, reduction of the negative impact of the environment and a number of other factors that negatively affect the functional state of humans, animals and poultry (Zhang & Singh, 2015; Salmanov & Muzyka, 2017; Yemtsev, 2022).

Ukraine is also part of the One Health strategy, although it is only at the beginning of harmonising its national food security requirements with EU standards and the scientific justification for the implementation of the HACCP (Hazard Analysis and Critical Control Point) system, which is based on the identification of critical control points and risk analysis of hygiene, safety of raw materials and livestock products. The livestock sector in Ukraine includes fish farming as a non-mainstream industry (Bryk, 2018; Garkavenko & Malimon, 2018).

The fisheries industry in Ukraine is part of the food sector and includes: catching and processing, reproduction and protection of fish stocks, breeding and rearing of commercial fish, breeding services, research and development, and a multi-level industry training system (Trofymchuk, 2021; Melnychenko & Bogadyorova, 2023). Ukraine has adopted the Strategy for the Development of the Fisheries Sector for the period up to 2030, which is key and determines the vectors of its development and the activities of fish processing enterprises in the country (Samofatova & Neveseliuk, 2020; Goncharova, 2023; Kotelevych, 2023).

Currently, all EU Member States are facing problems associated with the high prevalence of enteropathogenic strains of *E. coli*, pathogens of the genera *Salmonella* spp., *Enterococcus* spp. and *Campylobacter* spp. in raw materials and livestock products, including fisheries. As for the fishing industry, the infection is transmitted to humans through the consumption of contaminated fish and fish products (Park, 2012; Barrios, 2017). Scientists note that, according to the WHO, *E. coli* is a persistent pathogen. It is known that *E. coli* is capable of developing resistance to clinically important antibiotics – fluoroquinolones. The mechanism of action of fluoroquinolones is based on the specific inhibition of DNA gyrase in gram-negative microorganisms and topoisomerase IV in gram-positive bacteria (Shariati, 2022; Coba-Males, 2023). Fluoroquinolones are clinically important antibiotics with a broad antimicrobial spectrum of action covering gram-negative, gram-positive, aerobic, and anaerobic microorganisms. Scientists note that fluoroquinolones are bactericidally effective and can have a high bacterial effect in cases of antibiotic resistance of microorganisms to other antibiotic drugs. Fluoroquinolones have a rapid neutralising effect, high activity against intracellular pathogens, and high permeability to organs and tissues. Unlike beta-lactams, fluoroquinolones neutralise pathogens with minimal release of cellular components and prevent the release of large volumes of endotoxins from bacterial cells. Therefore, fluoroquinolones minimise the risk of complications (Lungu, 2022; Facchin, 2024).

In the fisheries industry, antibiotics are used to treat bacterial diseases of fish, which creates biological risks due to the possible deve-

lopment of acquired resistance in the pathogens of such diseases. Antibiotic-resistant strains of bacteria can be spread through fish and fish products, as fish is part of the food chain under the One Health concept. Contamination of fish and fish products with antibiotic-resistant bacteria can occur at all stages of the technological process, from farming to manufacturing of fish products.

Scientists and producers emphasise that the global increase in commercial fish farming is accompanied by the spread of bacterial infections that are treated with antibiotics added to fish feed. The scale of antibiotic use in fish farms is very large, and the consequences are unpredictable.

The aim of these studies was to determine the susceptibility of *E. coli* strains isolated from fish and fish products to clinically important representatives of fluoroquinolones of the II (ofloxacin, norfloxacin), III (levofloxacin) and IV (moxifloxacin) generations, to identify strains of *Escherichia coli* resistant to representatives of the indicator cephalosporins group: cefotaxime, ceftazidime and meropenem, to screen them to determine the probable production of beta-lactamases and carbapenemases (OXA-48 and OXA-48-like enzymes) and to confirm the production of extended-spectrum beta-lactamases (ESBL-enzymes) and AmpC-enzymes. We aimed to determine the prevalence of *E. coli* strains in raw materials and products of fish processing enterprises of Ukraine, which are fluoroquinolone-resistant and probably producing carbapenemase and producing beta-lactamase.

Materials and methods

The study was conducted at the State Research Institute for Laboratory Diagnostics and Veterinary and Sanitary Expertise (SRLVSE, Kyiv) and the Department of Microbiology and Virology of Bila Tserkva National Agrarian University (BNAU, Bila Tserkva) in 2023–2024.

Researched species of fish: silver carp (*Hypophthalmichthys molitrix*, Xenocyprididae), (Zhao, 2011); crucian carp (*Carasius carasius*, Cyprinidae), (Winfield & Nelson., 2012); common carp (*Cyprinus carpio carpio*, Cyprinidae), (Tsipas et al., 2008); common perch (*Perca fluviatilis*, Percadae), (Freyhof & Kottelat, 2008); silver hake (*Merluccius bilinearis*, Merlucciidae), (Carpenter, 2015); marble trout (*Salmo marmoratus*, Salmonidae); gilthead bream (*Sparus aurata*, Sparidae), (Roux, 1976); Caspian trout (*Salmo caspius*, Salmonidae), (Kessler, 1877); mackerel (*Scomber* sp., Scombridae), (Collette & Nauen, 1983); Atlantic horse mackerel (*Trachurus* sp., Carangidae), (John & MacGregor, 1966); herring (*Clupea* sp., Clupeidae), (Yabumoto & Nazarkin, 2018); blue mussel (*Mytilus edulis*, Mytilidae), (Mathiesen et al., 2016).

We studied 45 (13.4%) strains of *E. coli* isolated from 337 samples of fish and fish products from fish processing facilities in Ukraine. In particular, 4 (8.9% of the isolated) strains of *E. coli* from fresh fish samples, 7 (15.6%) strains of *E. coli* from chilled fish samples, 10 (22.2%) strains of *E. coli* from frozen fish samples, 4 (8.9%) strains of *E. coli* from salted fish samples, 7 (15.6%) strains of *E. coli* from smoked fish samples, 2 (4.4%) strains of *E. coli* from herring samples, 8 (17.7%) strains of *E. coli* from fish caviar samples and 3 (6.7%) strains of *E. coli* from seafood products.

Mueller Hinton agar M173 with a pH in the range of 7.2–7.4 was used for the study. The manufacturer is Himedia, the batch had been tested and standardised in accordance with the requirements of the CISI M6 document.

Antibiotic discs were used for sensitivity testing. To determine the susceptibility of the experimental strains of *E. coli* to fluoroquinolones, discs with antibiotic concentrations according to the latest Eucast recommendations were used: ofloxacin (5 μg) and norfloxacin (10 μg) – second-generation fluoroquinolones, levofloxacin (5 μg) – third-generation fluoroquinolones, and moxifloxacin (5 μg) – fourth-generation fluoroquinolones (The European Committee on Antimicrobial Susceptibility Testing. Breakpoint tables for interpretation of MICs and zone diameters. Version 14.0, 2024. www.eucast.org). To determine the susceptibility of the experimental *E. coli* strains to cephalosporins, discs with the antibiotics ceftazidime (10 μg), cefoxitin (30 μg), cefotaxime (5 μg), cefepime (30 μg), meropenem (10 μg), cefotaxime/clavulanic acid (30/10) were used. All antibiotic discs were manufactured by Himedia Laboratories Pvt. Limited, India.

The phenotypic method was used for regular internal quality control of the diffusion of discs with fluoroquinolones, cephalosporins and carbapenems according to Eucast recommendations (Eucast, Version 13.2, 2023). Measurement of growth inhibition zones was performed using a caliper compass. The results were interpreted in accordance with the latest valid version of Eucast according to the Breakpoint tables for the interpretation of the diameters of the zones of growth inhibition (Eucast, Version 14.0, 2024; The European Committee on Antimicrobial Susceptibility Testing. Breakpoint tables). According to the results of the control, the diameter of the growth inhibition zones under the action of the respective antibiotics on the test culture of *E. coli* ATCC 25922 was within the permissible values according to Eucast.

The phenotype method using discs with representatives of fluoroquinolones of the second ,third and fourth generations of ofloxacin, norfloxacin, levofloxacin and moxifloxacin was used to determine the resistance of the experimental *E. coli* strains to fluoroquinolones.

Primary phenotype screening for the probable production of extended-spectrum beta-lactamases (ESBLs), class C beta-lactamases (AmpC enzymes), carbapenemases (OXA-48 and OXA-48-like enzymes) was performed using discs with the indicator antibiotics cefotaxime (5 and 30 μg), ceftazidime (10 μg) and meropenem (10 μg) (Harkavenko, 2021).

Among the experimental *E. coli*, strains of *E. coli* likely to produce ESBL enzymes were identified and selected based on the size of the diameters of the zones of inhibition of culture growth in the ranges for cefotaxime (5 μg) < 20 mm (IF) and ceftazidime (10 μg) < 22 mm (IF) of one or both of them. The strains of experimental *Escherichia coli* likely to produce AmpC enzymes were determined by the diameter of the growth inhibition zone < 20 mm with cefotaxin (5 μg) and < 22 mm with ceftazidime (10 μg).

The phenotypic method using discs with meropenem (10 μg) was used to screen for the possible production of carbapenemase (OXA-48 and OXA-48-like enzymes) by the experimental strains of *E. coli* with a borderline sensitivity/moderate sensitivity value ≥ 22 mm to meropenem.

The combined discs and double disc methods confirmed the production of extended-spectrum acquired resistance enzymes (ESBL) by the experimental strains of *E. coli* selected during the primary screening.

The combined disc test was performed using ceftazidime (30 μg) alone and in combination with ceftazidime/clavulanic acid (30/10 μg). After thermostatic treatment at 37 ± 1 °C for 18–22 h, the results were considered positive if the difference in the diameters of the zones of growth inhibition of selected *E. coli* strains around the combined ceftazidime/clavulanic acid discs and the discs with ceftazidime was ≥ 5 mm.

The double disc test was performed using discs with cefotaxime (30 μg), ceftazidime (30 μg) and cefepime (30 μg) and discs with amoxiclav (10/10 μg) separately. The discs with indicator cephalosporins were placed at a distance of 20 mm from the discs with amoxiclav. Thermostatic treatment was performed at 37 ± 1 °C for 18–22 hours. ESBL production was confirmed by the expansion of the diameter of the zone of growth inhibition of *E. coli* experimental cultures from the disc with the indicator cephalosporin to the disc with amoxiclav – the "keyhole" effect.

Confirmation of the production of class C beta-lactamases (AmpC enzymes) by the experimental strains of *E. coli* selected during the primary screening was performed by the phenotype method using discs with cefoxitin (30 μg). The plates were incubated at 37 ± 1 °C for 18–22 h. The production of AmpC enzymes was indicated by the diameter of the zone of growth inhibition of experimental *E. coli* < 19 mm around the cefoxitin disc (Harkavenko, 2021).

The experimental strains of *E. coli* and the test culture of *E. coli* for growth control on MPA were thermostatted at 37 ± 1 °C for 24 h. Daily colonies of *E. coli* were resuspended in sterile saline to an optical density of 0.5 McFarland optical units (OU). The prepared suspensions of the respective *E. coli* strains were inoculated in 0.1 cm^3

on the surface of three plates with Mueller-Hinton agar (triplicate). After 15 min, discs with the corresponding antibiotics were placed on the agar surface (Harkavenko, 2021).

The test culture *E. coli* ATCC 25922 was used for quality control of diffusion of antibiotic discs, for setting up control of *E. coli* growth during studies on the sensitivity of selected *E. coli* strains to fluoroquinolones, as a control for phenotype screening and for setting up confirmatory methods for the production of acquired enzymes by experimental strains of *E. coli* (Eucast, Version 14.0, 2024).

Results

We studied 45 (13.4%) strains of *E. coli* isolated from 337 samples of fish and fish products from fish processing enterprises of Ukraine of different forms of ownership (private enterprises, individual entrepreneur).

Based on the analysis of the results of studies on the sensitivity to fluoroquinolone antibiotics, 14 (31.1% of the isolated strains) of the 45 *E. coli* strains were found to be resistant to one or two of the drugs used. The results of the study are presented in Table 1.

Table 1
Results of fluoroquinolone susceptibility testing of *E. coli* strains isolated from fish and fish products (n = 3)

Strains *E. coli*	Ofloxacin	Norfloxacin	Levofloxacin	Moxifloxacin
pEc1	20.3 ± 0.3^b	30.3 ± 0.3^a	28.3 ± 0.3^a	28.3 ± 0.3^a
pEc3	25.5 ± 0.3^a	30.3 ± 0.3^a	27.0 ± 0.7^b	24.7 ± 0.3^a
PEc17	25.6 ± 0.3^a	19.3 ± 0.3^b	19.3 ± 0.3^b	23.7 ± 0.7^a
pEc19	*d^b	34.0 ± 0.7^a	22.3 ± 0.3^c	24.7 ± 0.3^a
pEc20	d^b	35.3 ± 0.3^a	23.3 ± 0.3^c	25.7 ± 0.3^a
pEc22	19.7 ± 0.3^b	34.0 ± 0.7^a	19.7 ± 0.3^b	26.7 ± 0.3^a
pEc25	26.3 ± 0.3^a	30.7 ± 0.3^a	20.7 ± 0.3^b	25.7 ± 0.3^a
pEc26	25.0 ± 0.7^a	32.7 ± 0.3^a	21.3 ± 0.7^b	28.0 ± 0.7^a
PEc35	22.0 ± 0.7^c	19.7 ± 0.3^b	22.3 ± 0.3^c	23.3 ± 0.3^a
pEc43	26.3 ± 0.3^a	21.3 ± 0.3^a	20.3 ± 0.3^b	20.3 ± 0.3^b
pEc44	23.7 ± 0.3^c	31.0 ± 0.7^a	21.7 ± 0.3^c	20.7 ± 0.3^b
pEc45	31.0 ± 0.7^a	32.7 ± 0.3^a	19.7 ± 0.3^b	28.3 ± 0.3^a
pEc46	26.7 ± 1.0^a	33.3 ± 0.3^a	20.7 ± 0.3^b	27.3 ± 0.3^a
pEc47	20.3 ± 0.3^b	29.3 ± 0.3^a	21.7 ± 0.3^c	22.3 ± 0.3^a
Total: resistant	5	2	8	2
(% of allocated)	(11.1%)	(4.4%)	(17.8)	(4.4)

Note: range of Eucast growth inhibition zone diameters for ofloxacin: $\leq 24.0^a$ – sensitive, $> 22.0^b$ – resistant, $> 24.0 - < 22.0^c$ – moderately sensitive; for norfloxacin: $\leq 24.0^a$, $> 24.0^b$; for levofloxacin: $\leq 25.0^a$, $> 22.0^b$, $> 25.0 - < 22.0^c$; for moxifloxacin: $\leq 22.0^a$, $> 22.0^b$; *d^b – continuous growth of *E. coli* to discs.

The analysis of the test results showed resistance of *E. coli* bacteria to the representative of second generation fluoroquinolones – ofloxacin in 5 (11.1%) strains (pEC1, pEC19, pEC20, pEC22, pEC47). A representative of fluoroquinolones of the second generation, norfloxacin, had a higher bactericidal effect on the experimental cultures of *E. coli*. This is confirmed by resistance to this drug, which was detected only in 2 (4.4%) strains of *E. coli* (pEs17, pEs35). According to the study data, the lowest level of bactericidal efficacy among the fluoroquinolones used was inherent in the representative of the third generation – levofloxacin. Resistance to this drug was detected in 8 (17.8%) *E. coli* (strains pEc3, pEc17, pEc22, pEc25, pEc26, pEc43, pEc45, pEc46). The analysis of the results of studies to determine the susceptibility to the representative of the fourth generation fluoroquinolones – moxifloxacin – confirmed its bactericidal effect on the experimental cultures of *E. coli*. Resistance to this antibiotic was inherent in only 2 (4.4%) strains (pEC43, pEC44) of *E. coli*.

According to the results of studies on the detection of probable carbapenemase production in working strains of *E. coli* using discs with meropenem (10 μg), 6 (13.3%) cultures of *E. coli* (strains pEc8, pEc9, pEc10, pEc11, pEc12, pEc14) were suspected of possible production of OXA-48 and OXA-48-like enzymes at the borderline value of the diameters of growth inhibition zones at the level of ≥ 22 mm (sensitivity/moderate sensitivity range).

According to the results of the initial screening for the possible production of ESBL enzymes, 21 (46.7%) *E. coli* cultures (strains pES5, pES8, pES10, pES14, pES19, pES20, pES22, pES24, pES25, pES28, pES29, pES30, pES33, pES34, pES38, pEs43, pEs44, pEs45, pEs46, pEs47, pEs63) were detected , in which the diameters of the zones of growth inhibition under the action of indicator cephalosporins: cefotaxime (30 μg) was < 20 mm and ceftazidime (30 μg) was < 22 mm. These results indicated the possible production of extended-spectrum beta-lactamase (ESBL) by these experimental strains.

To confirm the production of ESBL enzymes by experimental *E. coli* strains among the probable beta-lactamase producers, the combined disc method was used (Table 2).

Table 2
Results of studies of *E. coli* strains for confirmation of ESBL production by the method of combined discs with ceftazidime (30 μg) and ceftazidime/clavulanic acid (30/10 μg, n = 3)

Strains *E. coli*	Difference between diameters of growth inhibition zones, mm	Product confirmation
pEc 5	8.0	**ESBL
pEc8	9.4	ESBL
pEc10	1.6	ESBL
pEc14	5.3	ESBL
pEc19	3.4	***(–)
pEc20	3.0	(–)
pEc22	3.7	(–)
pEc 24	3.7	(–)
pEc25	3.7	(–)
pEc28	3.4	(–)
pEc29	2.4	(–)
pEc30	4.3	(–)
pEc33	9.0	ESBL
pEc34	2.4	(–)
pEc 38	3.0	(–)
pEc43	3.7	(–)
pEc44	3.0	(–)
pEc45	3.7	(–)
pEc46	3.7	(–)
pEc47	3.6	(–)
pEc48	2.7	(–)
pEc56	4.3	(–)
pEc 63	3.3	(–)
Total: ESBL products confirmed (% of those tested)		5 (11.1%)

Note: **ESBL – confirmed production of ESBL enzymes, ***(–) – production of ESBL enzymes was not confirmed.

The production of ESBL enzymes by experimental *E. coli* strains was confirmed in 5 (11.1%) cultures of *E. coli* (strains pEc5, pEc8, pEc10, pEc14, pEc33) based on the results of studies using the method of combined discs with ceftazidime (30 μg) and ceftazidime/clavulanic acid (30/10 μg). The difference between the diameters of the zones of growth inhibition of ceftazidime in combination with clavulanic acid and ceftazidime alone varied at the level of ≥ 5 mm.

The accuracy of the data obtained on the production of ESBL beta-lactamase by the experimental strains of *E. coli* was evidenced by the results obtained using the double disc method with the indicator cephalosporins cefotaxime (30 μg), cefepime (30 μg), ceftazidime (30 μg), and amoxiclav (10/10 μg). According to the results of the study, in 5 (11.1%) of the experimental *E. coli* (strains pES5, pES8, pES10, pES14, pES33), a clearly defined expansion of the growth inhibition zones in the direction from the disc with the indicator cephalosporin to the disc with amoxiclav was observed – the "keyhole" effect (Table 3; Fig. 1).

The data obtained using the double disc method were consistent with the data from the experiments conducted using the combined disc method. Therefore, *E. coli* strains pEc5, pEc8, pEc10, pEc14, pEc33 are cultures in which the production of ESBL enzymes with extended spectrum of action has been confirmed.

According to the results of the initial screening of *E. coli* strains for possible production of AmpC enzymes, 6 (13.3% of the studied strains) (pEc20, pEc22, pEc25, pEc30, pEc33, pEc44) showed phenotypic resistance to ceftazidime (10 μg) and cefotaxime (5 μg), which indicated such a possibility. The results of further studies of *E. coli* strains suspected of producing AmpC-beta-lactamase with indicator cefoxitin (30 μg) showed that the diameters of the zones of in-

hibition of *E. coli* growth were less than 19.0 mm. The obtained results showed the resistance of *E. coli* strains to this indicator cephalosporin, which was confirmed by the production of AmpC enzymes.

Table 3
Results of studies of *E. coli* strains for confirmation of ESBL production by the double disc method using the "keyhole" effect (n = 3)

Strains *E. coli*	Cefotaxime (30 μg) and Amoxiclav (10/10 μg)	Cefepime (30 μg) and Amoxiclav (10/10 μg)	Ceftazidime (30 μg) and Amoxiclav (10/10 μg)	Product confirmation
pEc 5	*a	a	a	ESBL
pEc8	a	a	a	ESBL
pEc10	a	a	a	ESBL
pEc14	a	a	a	ESBL
pEc19	c	c	c	**(–)
pEc20	c	c	c	(–)
pEc22	c	c	c	(–)
pEc 24	c	c	c	(–)
pEc25	c	c	c	(–)
pEc28	c	c	c	(–)
pEc29	c	c	c	(–)
pEc30	c	a	c	(–)
pEc33	a	a	a	ESBL
pEc34	c	b	b	(–)
pEc 38	c	b	c	(–)
pEc43	c	c	b	(–)
pEc44	c	c	c	(–)
pEc45	c	b	c	(–)
pEc45	c	c	c	(–)
pEc47	*b	c	c	(–)
pEc48	c	b	c	(–)
pEc56	c	c	c	(–)
pEc 63	b	c	c	(–)
Total: *E. coli* strains producing ESBL (% of strains tested)				5 (11.1%)

Note: *a – the "keyhole" effect is clearly expressed; *b – the "keyhole" effect is weakly expressed; *c – the "keyhole" effect is not expressed; **(–) – ESBL enzyme production was not confirmed.

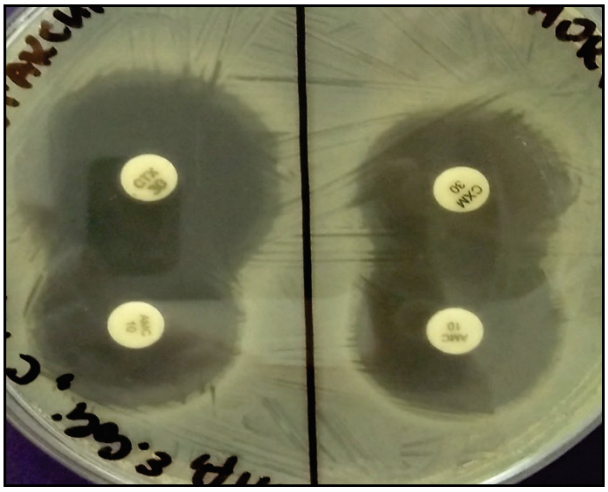

Fig. 1. Visualisation of the results of ESBL product validation studies using the double discs ("keyhole" effect)

Discussion

Fluoroquinolones are clinically important antibiotics. The presence of several fluorine atoms in the fluoroquinolone molecule affects the bactericidal activity of the drugs and their rapid pharmacokinetic action, which leads to effective bactericidal activity. A number of scientists argue that a decrease in sensitivity to one of the fluoroquinolones indicates resistance to other fluoroquinolones (Iakovlieva, 2022).

According to the results of our studies, this opinion of the authors was only partially confirmed in 3 strains of *E. coli* (pEC17, pEC22, pEC43). High rates of intra-group resistance were simultaneously demonstrated by: strain pEc17 to norfloxacin and levofloxacin, strain pEc22 to ofloxacin and levofloxacin, and strain pEc43 to levofloxacin and moxifloxacin. According to the results of our studies, the lowest bactericidal activity against the tested *E. coli* was found for levofloxacin. It is a representative of the third generation fluoroquinolones and should have high bactericidal activity against a wide range of pathogens. In our experiments, resistance to levofloxacin was inherent in 8 (17.8%) strains of experimental *E. coli*.

Data from scientists show the widespread prevalence of fluoroquinolone-resistant *E. coli* strains and confirm their particular resistance to ofloxacin, which was detected in 27.0% of cases among the studied *E. coli* isolates (Ashpur, 2022). According to the results of our studies, resistance to this drug was detected in 11.1% of *E. coli* strains among those studied.

A similar trend is observed in human medicine. According to the Center for Public Health of the Ministry of Health of Ukraine, based on the results of recent studies of strains of microorganisms isolated in Ukraine, among all strains of *E. coli*, resistance to ofloxacin was 76.2%, and to levofloxacin – 85.0% (Information bulletin on the results of the study of strains of microorganisms resistant to antimicrobial drugs isolated in Ukraine in 2024. State Institution "Centre for Public Health of the Ministry of Health of Ukraine" No. 04–09.22.1/5006/23 dated 09/11/2023; 248197DDF AB977E 5040000007 4C60F0188444304).

Researchers provide evidence that representatives of Enterobacteriaceae, *E. coli* in particular, producing ESBL beta-lactamases, are a serious global problem (Wilson, 2019; Bush & Bradford, 2020).

Using phenotypic methods for detecting ESBL-producing *E. coli* and genetic research methods, scientists have confirmed the stepwise evolution of ESBL-producing *E. coli* with the high-risk clone ST131 (Banerjee & Johnson, 2014; Ghotaslou, 2018). In this regard, the authors emphasise that the use of carbapenems for the treatment of bacterial infections is effective (Ghotaslou, 2018; Peirano & Pitout, 2019). In turn, another group of researchers has recently noted an increase in carbapenem resistance among *Klebsiella* and *E. coli* isolates and warned of low or no sensitivity to ertapenem in most *E. coli* isolates (Baroud, 2013; Livermore, 2019; Polishchuck, 2020). Scientists report that due to the continuing selective pressure for the use of beta-lactam antibiotics, the number of pathogens with beta-lactamase production is increasing (Prevar, 2018; Perez & Bonomo, 2019).

According to the results of our studies, carbapenem resistance has been identified in many cases among *E. coli* strains, especially those producing the beta-lactamase ESBL. According to our test results, 6 (13.3%) of the tested *Escherichia coli* strains were suspected of producing OXA-48 and OXA-48-like enzymes.

Other scientists, based on the results of tests, claim that resistance to carbapenems in Enterobacteriaceae, and in *E. coli* in particular, is associated with the overproduction of ESBL and AmpC enzymes (Dirar, 2020; Facchin, 2024).

According to the results of our studies, such features of the simultaneous production of ESBL enzymes with probable carbapenemase production were found in 4 (8.8%) of the tested *E. coli* strains.

Scientists have reported cases in which the production of AmpC enzyme can mask the production of ESBL enzyme in *E. coli* isolates. This fact was confirmed after the application of genetic research methods, which revealed ESBL genes along with AmpC enzymes.

Scientists report that representatives of Enterobacteriaceae, including *E. coli*, producing carbapenemase (OXA-48 and OXA-48-like enzymes) were first isolated in humans, and only later they were isolated in animals. Therefore, the authors emphasise the need for systematic monitoring to assess the prevalence of carbapenemase-producing *E. coli* in humans and animals at the global level (Pulss, 2018).

According to the results of our studies of fish and fish products, probable production of OXA-48 and OXA-48-like enzymes was detected in 13.3% of cases, production of ESBL enzymes in 11.1% and AmpC enzymes in 13.3% of cases among the studied *E. coli* strains. Given the data obtained, we emphasize the feasibility of expanding monitoring of *E. coli* studies for sensitivity to antibiotics of different groups, detection of acquired resistance enzymes of *Escherichia* isolated from facilities not only of the main livestock industries,

but also additional ones, in particular in fish processing as a component of the food chain.

Conclusion

The results of our studies provide an idea of the prevalence of fluoroquinolone-resistant *E. coli* strains isolated from fish and fish products samples in Ukraine. Resistance to fluoroquinolones of the second, third and fourth generations was inherent in 14 (31.1%) *Escherichia* strains. 6 (13.3%) *E. coli* strains were found to be suspected of producing OXA-48 and OXA-48-like enzymes, 5 (11.1%) *Escherichia* strains were found to be ESBL-producing, and 6 (13.3%) *Escherichia* strains tested produced AmpC enzymes. The data obtained are a justification and indicate the need to improve the microbiological control system in the fish industry in accordance with the current State Strategy of Ukraine on the implementation of the state policy to curb the development of antimicrobial resistance (AMR) and reduce the risks of the formation and spread of such strains of microorganisms in livestock, and also in accordance with the National Action Plan to Combat Antimicrobial Resistance, by expanding monitoring studies to determine the sensitivity of isolated *Escherichia* isolates to antibiotics, identifying their probable production of beta-lactamases and carbapenemases.

We thank the organization Czech University of Life Sciences Prague, Grant for Multidisciplinary Research Teams under the AgriSci-UA Platform/Project name: Prevalence of antibiotic-resistant bacterial strains in fish, fish products and water bodies in Ukraine.

There is no conflict of interest between the authors of the article.

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