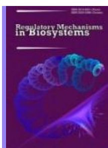

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Detection of *Escherichia coli* with acquired resistance to beta-lactams and carbapenems in animal and poultry feed in Ukraine

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The materials of the article are devoted to one of the directions for maintaining the biological safety of raw materials and livestock products through ensuring the safety of animal and poultry feed in accordance with the provisions of the global "One Health" concept. *Escherichia coli* strains isolated from samples of animal and poultry feed, when tested for susceptibility to cephalosporin antibiotics, revealed one of the hazardous factors – the production of acquired resistance enzymes, namely beta-lactamases and carbapenemases. During microbiological examination of 382 samples of mineral, organic, and artificial feed for animals and poultry, 21 (5.5% of the tested samples) *E. coli* cultures were isolated and identified. In 13 cases (61.9% of the isolates), the highest level of contamination with *Escherichia* was detected in compound feed, bran, meal, and oilcake samples. In 6 cases (28.6%), contamination was detected in concentrated feed (feed grain), and in 2 cases (9.5%) in premixes. The article presents the results of screening 21 experimental *E. coli* strains to identify those likely to produce extended-spectrum beta-lactamases (ESBL) and class C beta-lactamases (AmpC enzymes) using the disk diffusion method with indicator cephalosporins – cefotaxime (5 µg) and ceftazidime (10 µg). Experimental strains with inhibition zone diameters below the EUCAST established breakpoints (<20 and <22 mm, respectively) were selected. Such strains raised suspicion of possible production of acquired resistance enzymes. The production of extended-spectrum beta-lactamases (ESBL) was confirmed in five *E. coli* experimental strains (Ec18, Ec31, Ec53, Ec57, Ec64) using double-disk synergy and combination disk diffusion methods. Analysis of experimental results also confirmed the production of AmpC beta-lactamases in three *E. coli* strains (Ec31, Ec53, Ec69). These data were verified using the disk diffusion method with the indicator cephalosporin cefoxitin (30 µg), based on comparison with the EUCAST breakpoint of <19 mm for this antibiotic. The article also presents data on the detection of carbapenemase production using the primary disk diffusion method with meropenem (10 µg), an alternative method with ertapenem (10 µg), and an additional method using meropenem (20 µg) and temocillin (30 µg). According to the analysis of test results, no carbapenemase-producing *E. coli* strains (including OXA-48 and OXA-48-like enzymes) were identified among the tested isolates. The obtained data provide experimental justification for including microbiological testing of feed industry products in Ukraine in the State Monitoring Program for determining antimicrobial susceptibility of escherichial isolates contaminating animal and poultry feed. This would enable detection and confirmation of beta-lactamase and carbapenemase production in accordance with the National Action Plan to Combat Antimicrobial Resistance and contribute to reducing the risks of dissemination of such microorganisms in animal husbandry for the protection of human and animal health.

Keywords: mineral feed; organic feed; artificial feed; compound feed; meal; oilcake; feed grain; premixes; *E. coli*; acquired resistance; beta-lactamases; ESBL enzymes; AmpC enzymes; carbapenemases; OXA-48 and OXA-48-like enzymes.

Introduction

Animal and poultry feed and their administration are a critically important link in the global "One Health" concept and a strategic resource for the biological safety of Ukraine's livestock and poultry sectors. Feed directly affects all three components of this concept: the health of animals and poultry, human health, and the state of the environment. The main priority directions of the global "One Health" strategy include maintaining the health of humans, animals, and poultry while ensuring the production of high-quality and safe products. Meeting these requirements affects life expectancy, productivity, disease resistance, reduction of adverse environmental impacts, and a number of other factors that negatively influence the functional state of humans, animals, and poultry (Salmanov, 2017; Richard, 2022; Berman, 2023). Therefore, feed is one of the key components of a country's biosecurity measures (Kushnir, 2021; Overkovska, 2021; Manga, 2022).

The feed industry in Ukraine produces mineral, organic, and artificial feeds for animals and poultry. Organic feeds include plant-based products: concentrated feeds (feed grain), roughage (hay, straw, corn stalks), succulent feeds (green mass, silage, haylage, root crops), and plant materials with a high content of vitamins (herbal meal, seaweed meal). Animal-based feeds include skimmed milk, meat-and-bone meal, and by-products. Food and processing industry wastes include residues from elevator, milling, oil, starch-molasses, and fermentation production. Mineral feeds include salt, shell, and chalk. Artificial feeds comprise amino acids, vitamins, and premixes (Avercheva, 2021; Kuzmenko, 2021; Sklyar, 2024). Animal and poultry feeds serve as raw materials containing nutrients in forms accessible for absorption. They should not negatively affect the health of animals and poultry, reduce productivity, lower the quality of raw materials and livestock products, or create biological risks due to infections or contamination of feeds by bacterial pathogens. Therefore, global "One Health" strategies and the Global Health Security Agenda address the most pressing issues of feed and livestock product safety. The priority goals of the "One Health" strategy include maintaining human, animal, and poultry health while producing safe and high-quality products. Compliance with these requirements affects life expectancy, productivity, disease resistance, and reduces the negative impact of environmental factors on the functional state of humans, animals, and poultry (Collignon, 2019; Ayshpur, 2022; Stetsko, 2023). Ukraine is

also aligned with these strategies. According to the "One Health" concept, animal and poultry feed is a fundamental component of the food chain. Through livestock and poultry products, humans are also participants in this food chain. Therefore, Ukraine's feed base has a direct connection to this strategy. Problems related to the biosecure production of animal and poultry feeds are compounded by contamination with opportunistic and pathogenic microorganisms, including zoonotic agents. This creates a risk of infectious disease outbreaks (Shust, 2022; Chechet, 2023; Facchin, 2024). However, an even greater danger lies in the resistance of contaminant microorganisms to antimicrobial agents and their ability to develop acquired resistance to beta-lactams and carbapenems (Andersen, 2015; Rawson, 2017; Volkova, 2019). In Ukraine, within the framework of the "One Health" strategy, monitoring of raw materials and products across the entire agro-industrial sector is being carried out: data on the compliance of agricultural raw materials and products with microbiological criteria are collected, analyzed, and systematized (Bao, 2013; Salmanov, 2017; Peretyatko, 2022). Currently, researchers report a trend of increasing frequency of bacterial diseases linked to animal and poultry feed (Veklenko, 2020; Ayshpur, 2022; Peretyatko, 2022).

At the same time, an indisputable fact is the rising level of antibiotic resistance among bacterial microorganisms isolated from animal and poultry feeds, including acquired resistance to beta-lactams and carbapenems. This poses a threat to food safety for humans and is therefore a high-priority global issue (Perez, 2019; Van Boeckel, 2019; Romanyuk, 2020). Ukraine has implemented a National Action Plan to combat antimicrobial resistance in accordance with the Global Strategy of the World Health Organization (WHO) to contain antimicrobial resistance. However, such monitoring studies do not currently extend to the country's feed production sector.

The aim of this study was to determine the susceptibility of *E. coli* strains isolated from various types of animal and poultry feed to indicator cephalosporins – cefotaxime (5 µg) and ceftazidime (10 µg) – and to carbapenems – meropenem (10 µg); to identify *E. coli* strains resistant to these antibiotic groups; to screen them for suspected production of ESBL- and AmpC-type beta-lactamases and carbapenemases (OXA-48 and OXA-48-like enzymes); and to conduct confirmatory studies on the production of acquired resistance enzymes by the selected *E. coli* strains.

Materials and methods

The study was conducted during 2023–2024 at the Institute of Animal Biology of the National Academy of Agrarian Sciences of Ukraine (IAB NAAS, Lviv), the State Research Institute for Laboratory Diagnostics and Veterinary-Sanitary Expertise (SRILDVSE, Kyiv), and Bila Tserkva National Agrarian University (BTNAU, Bila Tserkva).

Experimental trials were carried out on 21 (5.5% of tested samples) *E. coli* strains previously isolated and identified from 382 samples of mineral, organic, and artificial animal and poultry feeds. Specifically, 2 (9.5%) experimental *E. coli* strains were isolated from artificial feeds – premixes; 6 (28.6%) strains from concentrated feeds (feed grain); 7 (33.3%) strains from compound feed and bran; and 6 (28.6%) strains from meals and oilcakes – by-products of oil and elevator processing industries.

Mueller-Hinton Agar (M173) with a pH range of 7.2–7.4 was used for the experiments. The medium was supplied by HIMEDIA, batch verified and standardized according to CISI–M6 requirements.

To determine the susceptibility of the *E. coli* strains to cephalosporins, discs containing the following antibiotics were used: ceftazidime (10 µg), cefoxitin (30 µg), cefotaxime (5 µg), cefepime (30 µg), meropenem (10 µg), cefotaxime/clavulanic acid (30/10 µg), and ceftazidime/clavulanic acid (30/10 µg). All antibiotic discs were produced by Himedia Laboratories Pvt. Limited, India.

Escherichia coli ATCC 25922 was used as a quality control for disk diffusion, to control the growth of *E. coli* in susceptibility testing with indicator cephalosporins (EUCAST, Version 15.0, 2025. www.eucast.org), and as a negative control for the absence of acquired extended-spectrum beta-lactamase (ESBL) production.

The test culture *Klebsiella pleuropneumoniae* ATCC 700603, which produces ESBL enzymes and is used according to EUCAST recommendations, was employed as a positive control for the detection of acquired extended-spectrum beta-lactamases (ESBLs).

Regular internal quality control of disk diffusion with cephalosporins and carbapenems was performed using the test culture *E. coli* ATCC 25922, in accordance with current EUCAST requirements (EUCAST, Version 13.2, 2023). The diameters of inhibition zones of the test culture *E. coli* ATCC 25922 were measured, and the results were interpreted according to the latest EUCAST version using the breakpoint tables for zone diameter interpretation (EUCAST, Version 15.0, 2025; The European Committee on Antimicrobial Susceptibility Testing. Breakpoint tables. www.eucast.org). The results of disk diffusion quality control showed that the inhibition zone diameters for the respective indicator cephalosporins and carbapenems on the test culture *E. coli* ATCC 25922 were within the acceptable ranges defined by the current EUCAST version. Therefore, these disks were approved for use in experiments.

Cultures of the experimental *E. coli* strains and control test cultures *E. coli* ATCC 25922 and *K. pleuropneumoniae* ATCC 700603 were grown on meat-peptone agar (MPA) at 37.0 ± 1.0 °C for 24 h. Daily colonies of *E. coli* were collected using a sterile bacteriological loop and resuspended in sterile physiological saline to an optical density of 0.5 McFarland units (McF).

Each prepared suspension of experimental *E. coli* strains and test cultures was inoculated in 0.1 cm³ aliquots and evenly spread across the surfaces of five Mueller-Hinton agar plates (fivefold replication) and one plate of *E. coli* ATCC 25922 for growth control on MPA. After 15 minutes, antibiotic discs were placed on the agar surface (Harkavenko et al., 2021). The plate with the test culture was used to confirm the growth intensity of *E. coli*, and antibiotic discs were not applied on its surface. All plates were incubated at 37.0 ± 1.0 °C for 18–20 h. Results were recorded by measuring the diameters of inhibition zones for the experimental *E. coli* cultures, and interpretation was performed according to the latest EUCAST breakpoint tables for disk diffusion (EUCAST, Breakpoint tables for interpretation of MICs and zone diameters, Version 14.0, valid from 2024-01-01. www.eucast.org).

Primary phenotypic screening using the disk diffusion method was conducted to detect potential production of extended-spectrum beta-lactamases (ESBLs), class C beta-lactamases (AmpC enzymes), and carbapenemases (OXA-48 and OXA-48-like enzymes) using indicator cephalosporin discs: cefotaxime (5 µg and 30 µg), ceftazidime (10 µg), and meropenem (10 µg) (Harkavenko et al., 2021). Experimental *E. coli* strains suspected of producing acquired ESBL or AmpC enzymes were selected based on inhibition zone diameters according to EUCAST: cefotaxime (5 µg) < 21 mm and ceftazidime (10 µg) < 22 mm.

Screening for potential carbapenemase production (OXA-48 and OXA-48-like enzymes) was performed using meropenem discs (10 µg), with EUCAST breakpoint ≤ 22 mm.

Confirmatory disk diffusion methods, including combined disc and double-disc synergy tests, were used to verify production of acquired extended-spectrum beta-lactamases (ESBLs) by *E. coli* strains flagged as suspicious in primary screening.

Discs with ceftazidime (30 µg) alone and in combination with ceftazidime/clavulanic acid (20/10 µg), cefotaxime (30 µg) alone and with clavulanic acid (20/10 µg), and cefepime (30 µg) alone and with clavulanic acid (20/10 µg) were applied. Plates were incubated at 37 ± 1 °C for 18–22 h. Results were considered positive if the difference in inhibition zone diameters between single and combined discs was ≥ 5 mm. The double-disk diffusion test was performed using disks containing cefotaxime (30 µg), ceftazidime (30 µg), and cefepime (30 µg), along with separate disks containing amoxiclav (20/10 µg). The indicator cephalosporin disks were placed at a distance of 20 mm from the amoxiclav disks. Incubation was carried out at 37 ± 1 °C for 18–22 h. ESBL production was confirmed by the expansion of the inhibition zone of the experimental *E. coli* cultures from the indicator cephalosporin disk toward the amoxiclav disk, forming a clearly defined irregularly shaped ellipse – the "keyhole effect".

The production of class C beta-lactamases (AmpC enzymes) by *E. coli* strains suspected from the screening was confirmed phenotypically using disks containing cefoxitin (30 µg). Incubation was carried out at 37 ± 1 °C for 18–22 h. AmpC production was confirmed when the diameters of the inhibition zones of the experimental *E. coli* strains were <19 mm, in accordance with the established EUCAST breakpoints (Harkavenko, 2021).

Values are presented as the arithmetic mean (x) ± standard deviation (SD). Differences between control and experimental groups were considered statistically significant at $P < 0.05$ (Hoiko, 2004).

Results

According to the obtained data from the screening, exposure to cefotaxime (5 µg) revealed that the mean inhibition zone diameters of 7 (33.3%) experimental *E. coli* strains (Ec18, Ec31, Ec49, Ec53, Ec57, Ec64, Ec69) were below the established EUCAST breakpoint of < 20 mm. The inhibition zone diameters of these experimental *E. coli* strains ranged from 17.6 ± 0.2 to 19.4 ± 0.4 mm, indicating a potential production of beta-lactamases (Table 1).

Table 1
Screening results for *E. coli* strains potentially producing acquired beta-lactamases (inhibition zone diameter, mm, n = 21, x ± SD)

No.	Names of strains of experimental *E. coli*	°Ø for the action of cefotaxime (5 µg), mm	Ø for the action of ceftazidime (10 µg), mm
1	Ec15	27.8 ± 0.2 ^{d}	35.2 ± 0.2 ^{d}
2	Ec16	28.0 ± 0.3 ^{d}	24.2 ± 0.2 ^{d}
3	Ec18	▪19.4 ± 0.2 ^{c}	▪10.4 ± 0.4 ^{d}
4	Ec21	23.8 ± 0.2 ^{d}	26.2 ± 0.2 ^{d}
5	Ec27	26.0 ± 0.4 ^{d}	28.0 ± 0.3 ^{d}
6	Ec31	▪17.6 ± 0.2 ^{d}	▪15.2 ± 0.4 ^{d}
7	Ec42	▪24.2 ± 0.4 ^{d}	▪27.0 ± 0.5 ^{d}
8	Ec49	▪19.4 ± 0.2 ^{d}	▪19.4 ± 0.2 ^{d}
9	Ec53	18.6 ± 0.4 ^{c}	19.8 ± 0.4 ^{d}
10	Ec55	26.0 ± 0.5 ^{d}	24.4 ± 0.2 ^{d}
11	Ec57	▪19.4 ± 0.2 ^{b}	▪19.8 ± 0.4 ^{d}
12	Ec58	25.2 ± 0.2 ^{d}	23.4 ± 0.5 ^{c}
13	Ec59	26.2 ± 0.4 ^{d}	25.4 ± 0.2 ^{d}
14	Ec61	24.0 ± 0.3 ^{d}	22.6 ± 0.2 ^{d}
15	Ec64	▪19.4 ± 0.4 ^{b}	▪19.8 ± 0.4 ^{d}
16	Ec65	26.2 ± 0.4 ^{d}	28.0 ± 0.3 ^{d}
17	Ec66	28.4 ± 0.2 ^{d}	29.8 ± 0.4 ^{d}
18	Ec67	26.2 ± 0.4 ^{d}	26.0 ± 0.3 ^{d}
19	Ec68	24.2 ± 0.4 ^{d}	28.2 ± 0.2 ^{d}
20	Ec69	▪19.4 ± 0.2 ^{b}	▪19.6 ± 0.5 ^{c}
21	Ec70	24.2 ± 0.4 ^{d}	26.2 ± 0.4 ^{d}

Note: °Ø – diameters of culture growth inhibition zones; ^{b} – $P < 0.05$; ^{c} – $P < 0.01$; ^{d} – $P < 0.001$, compared to the EUCAST breakpoint criteria for cefotaxime (5 µg) < 20 mm, for ceftazidime (10 µg) < 22 mm; ▪ – *E. coli* strains suspected of producing beta-lactamases (ESBL and AmpC enzymes) in accordance with the EUCAST breakpoint criteria.

These *E. coli* strains were selected for testing to confirm their production of beta-lactamases. Screening with ceftazidime (10 µg) indicated a potential production of beta-lactamases in 7 (33.3%) *E. coli* cultures (strains Ec18, Ec31, Ec49, Ec53, Ec57, Ec64, Ec69), as the mean inhibition zone diameters of these experimental cultures ranged from 10.4 ± 0.4 to 19.8 ± 0.4 mm, below the EUCAST breakpoint of < 22 mm.

To confirm the production of acquired extended-spectrum beta-lactamases (ESBLs) using the double-disc method, results from positive and negative controls with test cultures recommended by EUCAST were considered. The positive control using *K. pleuropneumoniae* ATCC 700603 confirmed the proper setup of the experiment and the validity of the results. This test culture produces ESBLs, and upon interaction with indicator cephalosporins, formed a distinct deformed ellipse – the "keyhole" effect – showing an expanded inhibition zone toward the amoxicillin-clavulanic acid disc (10 µg).

The *E. coli* ATCC 25922 test culture does not produce ESBLs, and therefore the "keyhole" effect was absent. The results of the confirmatory double-disc method for ESBL production are summarized in Table 2.

Table 2
Confirmation of ESBL production by experimental *E. coli* strains using the double-disc method (n = 7)

Experimental strains *E. coli*	Cefotaxime (30 µg) and amoxicillin-clavulanic acid (20/10 µg)	Cefepime (30 µg) and amoxicillin-clavulanic acid (20/10 µg)	Ceftazidime (30 µg) and amoxicillin-clavulanic acid (20/10 µg)
Ec18	(+ ▪)	(+ ▪)	(+ ▪)
Ec31	(+ ▪)	(+ ▪)	(+ ▪)
Ec49	±	±	-
Ec53	(+ ▪)	(+ ▪)	(+ ▪)
Ec57	(+ ▪)	(+ ▪)	(+ ▪)
Ec64	(+ ▪)	(+ ▪)	(+ ▪)
Ec69	-	-	±
*Test culture *E.coli* ATCC 25922	-	-	-
**Test culture *Kl. pleuropneumoniae* ATCC 700603	(+ ▪)	(+ ▪)	(+ ▪)

Note: * – negative control with *E. coli* ATCC 25922 test culture according to EUCAST recommendations, which does not produce ESBLs; ** – positive control with *K. pleuropneumoniae* ATCC 700603 test culture according to EUCAST recommendations, which produces ESBLs; (+ ▪) – *E. coli* strains with a clearly expressed "keyhole" effect, confirming ESBL production; ± – *E. coli* strains with a weakly expressed "keyhole" effect; – – *E. coli* strains without any indication of the "keyhole" effect.

Based on the results of the double-disc method experiment, it was established that among the 7 (33.3% of the experimental) suspected *E. coli* strains, 5 (23.8 %) strains – Ec18, Ec31, Ec53, Ec57, and Ec64 – produced extended-spectrum beta-lactamases (ESBLs). The results were confirmed by clear inhibition zones of the experimental *E. coli* cultures, forming distinctly deformed ellipses – the "keyhole" effect. Using the combined-disc method to confirm ESBL production, the consistency of the positive control with *K. pleuropneumoniae* ATCC 700603 and the negative control with *E. coli* ATCC 25922, according to EUCAST recommendations, confirmed the correct setup of the experiment and the validity of the obtained results (Table 3). Among the 7 (33.3% of the experimental) *E. coli* strains suspected of

beta-lactamase production, 5 (23.8% of the experimental) strains – Ec18, Ec31, Ec53, Ec57, and Ec64 – showed differences between inhibition zone diameters exceeding the EUCAST breakpoint of ≥ 5 mm. Thus, ESBL production was confirmed in these suspected experimental *E. coli* strains after exposure to individual indicator cephalosporins: ceftazidime (30 µg), cefotaxime (30 µg), and cefepime (30 µg), as well as separately to amoxicillin-clavulanic acid (20/10 µg), based on the differences in inhibition zone diameters.

Regarding the 7 (33.3% of the experimental) *E. coli* strains suspected of producing AmpC beta-lactamases, 3 (14.3% of the experimental) strains (Ec31, Ec53, and Ec69) after exposure to cefotaxime discs (30 µg) showed mean inhibition zone diameters ranging from confluent growth to 13.3 ± 0.3 mm for strain Ec69. These values were below the EUCAST breakpoint for cefotaxime > 19 mm. Based on these results, AmpC production was confirmed in the indicated experimental *E. coli* strains (Table 4).

Table 3
Results of the combined-disc method for confirming ESBL production by experimental *E. coli* strains suspected of potential ESBL production (inhibition zone diameter, mm, n = 7; x ± SD)

No.	Names of suspected strains *E. coli*	°Ø Upon exposure to cefotaxime (30 µg)	Ø Upon exposure to cefotaxime/clavulanic acid (30 µg), mm	Difference in Ø, mm	Ø upon exposure to ceftazidime (30 µg)	Ø upon exposure to ceftazidime/clavulanic acid (30 µg), mm	Difference in Ø, mm
1	Ec18	25.2 ± 0.4[d]	31.6 ± 0.2[d]	6.4 ▪	16.4 ± 0.5[d]	23.0 ± 1.0[c]	6.6 ▪
2	Ec31	confluent growth	confluent growth	P ▪	23.0 ± 0.3[d]	28.5 ± 0.4[d]	5.5 ▪
3	Ec49	22.0 ± 0.4[d]	25.8 ± 0.5[d]	3.8	23.8 ± 0.5[d]	26.2 ± 0.4[d]	2.4
4	Ec53	confluent growth	confluent growth	P ▪	23.8 ± 0.4[d]	31.0 ± 0.3[d]	7.2 ▪
5	Ec57	20.0 ± 0.4[a]	28.0 ± 0.3[d]	8.0 ▪	22.8 ± 0.5[d]	30.2 ± 0.4[d]	7.4 ▪
6	Ec64	28.0 ± 0.4[d]	36.0 ± 0.4[d]	8.0 ▪	24.4 ± 0,4[c]	32.8 ± 0.4[d]	8.4 ▪
7	Ec69	13.0 ± 0.3[b]	15.2 ± 0.4[d]	2.2	24.4 ± 0.2[d]	26.6 ± 0.4[d]	2.2
			Controls				
*Test culture *E.coli* ATCC 25922		30.0	33.0	3.0	28.0	30.0	2.0
**Test culture *K. pleuropneumoniae* ATCC 700603		28.0	35.0	7.0 ▪	29.0	37.0	8.0 ▪

Note: * – negative control for result quality according to EUCAST recommendations; ** – positive control for result quality according to EUCAST recommendations; °Ø – inhibition zone diameters of the culture; [a] – P < 0.1; [b] – P < 0.05; [c] – P < 0.01; [d] – P < 0.001, compared with the EUCAST established breakpoints for cefotaxime (30 µg) < 17 mm and ceftazidime (30 µg) < 19 mm; ▪ – experimental *E. coli* strains producing ESBLs with a difference in inhibition zone diameters, according to EUCAST, ≥ 5 mm.

Table 4
Results of testing suspected *E. coli* strains for AmpC beta-lactamase production (inhibition zone diameter, mm, n = 21, x ± SD)

No.	Name of suspected *E. coli* strains	°Ø Upon exposure to cefotaxime (5 µg), mm	Ø upon exposure to ceftazidime (10 µg), mm	Ø Upon exposure to cefoxitin (30 µg), mm
1	Ec18	19.4 ± 0.2[c]	10.4 ± 0.4[d]	25.6 ± 0.2[d]
2	Ec31	17.6 ± 0.2[d] ▪	15.2 ± 0.4[d] ▪	confluent growth ▪
3	Ec49	19.4 ± 0.2[d]	19.4 ± 0.2[d]	22.8 ± 0.4[d]
4	Ec53	18.6 ± 0.4[b] ▪	19.8 ± 0.4[d] ▪	confluent growth ▪
5	Ec57	19.4 ± 0.2[c]	19.8 ± 0.4[d]	21.6 ± 0.5[d]
6	Ec64	19.4 ± 0.4[c]	19.8 ± 0.4[d]	29.0 ± 0.3[d]
7	Ec69	19.4 ± 0.2[c] ▪	19.6 ± 0.5[b] ▪	13.0 ± 0.3[d] ▪

Note: °Ø – inhibition zone diameters of the culture; [b] – P < 0.05; [c] – P < 0.01; [d] – P < 0.001, compared with the EUCAST established breakpoints: cefotaxime (5 µg) < 20 mm, ceftazidime (10 µg) < 22 mm, and cefoxitin (30 µg) < 19 mm; ▪ – *E. coli* strains producing AmpC beta-lactamases according to the established EUCAST breakpoints.

Table 5
Results of studies using the primary and alternative methods for detecting carbapenemase production (OXA-48 and OXA-48-like enzymes) in experimental *E. coli* strains (mm, n = 21; x ± SD)

No.	Name of experimental *E. coli* strains	°Ø by primary method for detection of acquired carbapenemase production with meropenem (10 µg), mm	Ø by alternative method for detection of acquired carbapenemase production with ertapenem (10 µg), mm
1	Ec15	26.4 ± 0.2[d]	25.8 ± 0.4[a]
2	Ec16	34.6 ± 0.4[d]	27.6 ± 0.2[d]
3	Ec18	32.6 ± 0.4[d] □	26.6 ± 0.2[d] □
4	Ec21	34.0 ± 0.3[d] □	26.8 ± 0.2[d] □
5	Ec27	38.4 ±0.2[d] □	32.8 ± 0.2[d] □
6	Ec31	30.4 ±0.2[d] □	25.8 ± 0.2[c] □
7	Ec42	36.4 ± 0.2[c] □	31.2 ± 0.6[d] □
8	Ec49	33.4 ± 0.9[d] □	26.8 ± 0.4[c] □
9	Ec53	35.2 ± 0.2[d] □	31.2 ± 0.6[d] □
10	Ec55	32.4 ± 0.2[d] □	28.0 ± 0.3[d] □
11	Ec57	32.6 ± 0.4[d] □	32.8 ± 0.9[d] □
12	Ec58	36.2 ± 0.2[d] □	31.0 ± 0.3[d] □
13	Ec59	35.8 ± 0.4[d] □	28.4 ± 0.4[d] □
14	Ec61	35.6 ± 0.4[d] □	31.6 ± 0.2[d] □
15	Ec64	33.8 ± 0.2[d] □	26.8 ± 0.2[d] □
16	Ec65	31.2 ± 0.2[d] □	28.4 ± 0.4[d] □
17	Ec66	28.4 ± 0.2[d] □	24.0 ± 0.4[a] □
18	Ec67	31.0 ± 0.6[d] □	28.2 ± 0.2[d] □
19	Ec68	26.2 ± 0.2[d] □	21.2 ± 0.6[d] □
20	Ec69	31.2 ± 0.5[d] □	27.6 ± 0.4[d] □
21	Ec70	28.8 ± 0.4[d] □	22.4 ± 0.2[d] □

Note: °Ø – inhibition zone diameters of the culture; [a] – P < 0.1; [b] – P < 0.05; [c] – P < 0.01; [d] – P < 0.001, compared with the EUCAST established breakpoints: meropenem (10 µg) < 22–27 mm, ertapenem (10 µg) < 25 mm; □ – experimental *E. coli* strains not producing carbapenemases (including OXA-48 and OXA-48-like enzymes) according to the established EUCAST breakpoints.

Thus, AmpC beta-lactamase production was confirmed in 3 (14.3% of the experimental) *E. coli* strains: Ec31, Ec53, and Ec69. To detect the production of acquired carbapenemases (OXA-48 and OXA-48-like enzymes) by the experimental *E. coli* strains, tests were conducted using primary, additional, and alternative phenotypic disc-diffusion methods according to EUCAST recommendations. The results of the primary and additional disc-diffusion methods are presented in Table 5.

Additionally, to identify carbapenemase-producing strains among the 21 experimental *E. coli* cultures, testing was conducted using an additional disk-diffusion method with meropenem (20 µg) and a representative of the penicillin group – temocillin (30 µg). Analysis of the results showed that after exposure to meropenem (20 µg), the inhibition zone diameters of all experimental *E. coli* cultures ranged from 28.6 ± 0.2 to 30.8 ± 0.7 mm. These values exceeded the EUCAST-established breakpoints for inhibition zone diameters (< 22–27 mm). After exposure to temocillin (30 µg), the diameters of inhibition zones for the *E. coli* cultures significantly exceeded the EUCAST breakpoint of < 11 mm for this antibiotic. Comparative analysis of the results confirmed the absence of acquired carbapenemase production (including OXA-48 and OXA-48-like enzymes). The results of these tests are presented in Table 6.

Table 6
Results of additional disk-diffusion testing to confirm carbapenemase production (including OXA-48 and OXA-48-like enzymes) by experimental *E. coli* strains (mm, n = 21; x ± SD)

No.	Names of the experimental *E. coli* strains	°Ø under the action of meropenem (20 µg), mm	Ø under the action of temocillin (30 µg), mm
1	Ec15	28.6 ± 0.2^{b} □	14.2 ± 0.6^{d}□
2	Ec16	29.0 ± 0.4^{a} □	13.6 ± 0.4^{d}□
3	Ec18	29.0 ± 0.4^{b} □	13.6 ± 0.4^{d}□
4	Ec21	30.0 ± 0.4^{d} □	12.4 ± 0.2^{d}□
5	Ec27	29.2 ± 0.5^{b}□	13.6 ± 0.4^{d}□
6	Ec31	29.2 ± 0.5^{b}□	12.8 ± 0.2^{d}□
7	Ec42	29.8 ± 0.2^{d}□	13.6 ± 0.4^{d}□
8	Ec49	30.8 ± 0.7^{c}□	14.4 ± 0.4^{d}□
9	Ec53	28.8 ± 0.2^{c}□	13.4 ± 0.5^{c}□
10	Ec55	28.6 ± 0.2^{b}□	15.4 ± 0.4^{d}□
11	Ec57	29.4 ± 0.4^{c}□	14.8 ± 0.2^{d} □
12	Ec58	30.2 ± 0.2^{d}□	13.8 ± 0.2^{d}□
13	Ec59	28.6 ± 0.2^{d}□	15.4 ± 0.2^{d}□
14	Ec61	28.8 ± 0.2^{c}□	12.4 ± 0.2^{d}□
15	Ec64	30.4 ± 0.2^{d}□	17.0 ± 0.3^{d}□
16	Ec65	32.2 ± 0.4^{d}□	17.2 ± 0.2^{d}□
17	Ec66	29.6 ± 0.4^{c}□	20.8 ± 0.4^{d}□
18	Ec67	29.4 ± 0.2^{d}□	20.2 ± 0.6^{d}□
19	Ec70	29.6 ± 0.7^{b}□	16.8 ± 0.4^{d}□
20	Ec69	29.2 ± 0.5^{b}□	14.4 ± 0.2^{d}□
21	Ec68	28.8 ± 0.2^{c}□	15.8 ± 0.4^{d}□

Note: °Ø – diameters of inhibition zones of the tested *E. coli* strains; ^{a} – P < 0.1; ^{b} – P < 0.05; ^{c} – P < 0.01; ^{d} – P < 0.001, compared with the established EUCAST breakpoint criteria for meropenem (20 µg) at < 25–27 mm and for temocillin (30 µg) at < 11 mm; □ – *E. coli* strains not producing carbapenemases (including OXA-48 and OXA-48-like enzymes) according to the established EUCAST breakpoint criteria.

Thus, in the conducted study on the detection of acquired resistance enzyme production among 21 experimental *E. coli* strains, the production of extended-spectrum beta-lactamases (ESBLs) was confirmed in 5 (23.8% of the tested) *E. coli* cultures: strains Es18, Es31, Es53, Es57, Es64; the production of class C beta-lactamases (AmpC enzymes) was confirmed in 3 (14.3% of the tested) *E. coli* cultures: strains Es31, Es53, Es69; and no carbapenemase producers (including OXA-48 and OXA-48-like enzymes) were detected among the tested *E. coli* strains.

Discussion

Scientists assert that modern medical and veterinary science and practice face an increasing challenge in determining the role of opportunistic microorganisms in the pathology of humans and animals. Members of epidemiologically significant risk groups include *E. coli*. *Escherichia coli* is a normal component of the microflora of humans and animals, but pathogenic strains, particularly enteropathogenic ones, can cause infectious diseases with diverse clinical manifestations (Bao, 2013). Researchers note that *E. coli* has gained particular importance due to the rapid and significant acquisition of antibiotic resistance determinants, especially to cephalosporins (Gupta, 2014; Veldman, 2014). High prevalence of antibiotic resistance has been detected among isolates of *E. coli* that are part of the normal microflora. This makes them a potential source of dissemination of resistance determinants within their own populations and to related bacterial species across different taxonomic groups (Kotsyuba, 2014; Voyda, 2014). The results of our studies align with these findings, confirming the rapid spread of cephalosporin-resistant *E. coli* strains in the environment and in agricultural raw materials and products.

Researchers report that members of the family Enterobacteriaceae, particularly *E. coli* producing ESBL beta-lactamases, represent a serious global problem (Bush, 2020; Dirar, 2020). It is noted that Enterobacteriaceae are an integral part of the biosphere (Qiulian, 2021). Due to their polybiotrophic nature and adaptive properties, they are widely distributed in the environment, in humans, animals, and plants. Plants are components of animal and poultry feed and of human food. Studies indicate that when ESBL-producing *E. coli* isolates were detected in samples of culinary herbs-such as parsley, ipomoea, acacia leaves, betel, basil, and other herbs – several isolates simultaneously produced AmpC enzymes (Veldman, 2014). Our findings are consistent with these results, as *E. coli* strains isolated from animal and poultry feeds were found to simultaneously produce both ESBL and AmpC enzymes. Using phenotypic methods to detect ESBL-producing *E. coli* and genetic research approaches, scientists have confirmed the stepwise evolution of ESBL-producing *E. coli* from the high-risk clone ST131 (Banerjee & Johnson, 2014; Ghotaslou, 2018). In this context, the authors emphasize that the use of carbapenems for the treatment of bacterial infections is effective in such cases (Ghotaslou, 2018; Peirano, 2019). The results of our study support these findings, as no *E. coli* strains contaminating animal and poultry feeds were found to produce carbapenemases. This may indicate the effectiveness of carbapenems for therapeutic purposes.

Conclusion

Microbiological methods were used to study 21 (5.5% of the tested samples) *E. coli* strains isolated from 382 samples of animal and poultry feed to detect acquired β-lactamases and carbapenemases as potential threats to feed safety. Feed is an integral component of the "food chain" and directly affects the quality and biosafety of raw ma-

terials and products in the livestock and poultry sectors. Therefore, feed biosafety is closely linked to the health of humans, animals, and poultry.

Screening using the disk-diffusion method revealed that among the 21 experimental *E. coli* strains, 7 (33.3% of the tested strains) were suspected of producing acquired extended-spectrum β-lactamases (ESBLs) and class C β-lactamases (AmpC enzymes). The production of extended-spectrum β-lactamases (ESBLs) was confirmed in 5 (23.8% of the tested strains) cultures – strains Es18, Es31, Es53, Es57, and Es64 – using double-disk and combined-disk methods. The production of class C β-lactamases (AmpC enzymes) was confirmed by the disk-diffusion method in 3 (14.3% of the tested strains) *E. coli* cultures (strains Es31, Es53, and Es69) using cefoxitin (30 µg) as the indicator antibiotic.

No experimental *E. coli* strain producing acquired carbapenemases (including OXA-48 and OXA-48-like enzymes) was detected using the primary method with meropenem (10 µg), the alternative method with ertapenem (10 µg), or the additional method with meropenem (20 µg) and temocillin (30 µg).

Future prospects of this research include developing a theoretical and practical basis to support the enhancement of the State Program for monitoring animal and poultry feed. This would involve identifying a broader spectrum of bacterial feed contaminants, studying their antibiotic resistance, and detecting their production of acquired resistance enzymes to address the prevention of zoonotic pathogen dissemination in the "food chain" according to the global "One Health" concept.

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The authors declare no conflict of interest.

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