

РОЗДІЛ 1. Емерджентні хвороби та Єдине здоров'я  
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Multidrug-resistant *Enterobacteriaceae* in veterinary practice: current challenges

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**Abstract.** Antimicrobial resistance in companion animals is of growing importance within the One Health approach due to close contact between humans and domestic animals and the risk of circulation of multidrug-resistant pathogens in households and veterinary clinics. The aim of the study was to evaluate the structure of clinically significant isolates of the *Enterobacteriaceae* family and the proportion of multidrug-resistant (MDR) strains in dogs and cats with clinical signs of bacterial infections in veterinary clinics in Kyiv in 2022–2025.

Wound material was collected with sterile swabs from the depth/area of active inflammation and placed in Amies transport medium (AMIES). Samples ( $n=63$ : feces -17 (27.0%) and swabs from purulent-inflammatory wounds - 46 (73.0%)) were transported at +2...+8 °C. Cultivation was performed on MacConkey agar and Endo medium with incubation at 35–37 °C for 18–24 hours; identification was performed using standard biochemical tests. Sensitivity to antimicrobial drugs was determined using the Kirby–Bauer method on Mueller–Hinton agar with interpretation according to EUCAST; ESBL was detected using the DDST method. MDR was defined as insensitivity to at least one drug in three or more classes. The data were summarized descriptively ( $n, \%$ ).

The study was conducted as a single-moment observational study. Of the 63 clinical samples selected, the dominant isolate was *Escherichia coli* - 44/63 (69.8%), while *Klebsiella* spp. accounted for 19/63 (30.2%). MDR was detected in 15/63 isolates (23.8%), i.e., almost every fourth isolate was characterized by multidrug resistance. The detection of *Klebsiella* spp. as the second most frequent group is also clinically significant, since representatives of this genus are often associated with clinically associated infections and are capable of accumulating resistance factors, which complicates treatment.

In veterinary clinics in Kyiv in 2023–2025, *E. coli* predominated among clinical isolates of *Enterobacteriaceae*, and the proportion of MDR was 23.8%. The recorded proportion of MDR indicates an increased risk of ineffective empirical antibiotic therapy and justifies the need for wider use of bacteriological testing and susceptibility testing for clinically significant cases, particularly in purulent-inflammatory lesions of the skin and soft tissues. The results confirm the need for regular local microbiological monitoring, enhanced infection control, and rational use of antimicrobial drugs in the treatment of companion animals.

**Keywords:** antimicrobial resistance, multidrug resistance, *Enterobacteriaceae*, *Escherichia coli*, *Klebsiella* spp., companion animals, veterinary clinics, One Health

The issue of antimicrobial resistance (AMR) in domestic animals is becoming increasingly relevant for several reasons: the constant use of antibacterial drugs in veterinary practice, close contact between small pet owners and their animals, and intensive microbial exchange within homes and veterinary clinics. Under these conditions, animals can act not only as a factor reflecting the circulation of resistant strains in the shared environment, but also as a potential reservoir and “bridge” for the transmission of multidrug-resistant enterobacteria between humans, animals, and the environment, making the problem cross-sectoral from a One Health perspective (Walther, 2023; Menezes, 2024; Monteiro, 2025).

The transmission of multidrug-resistant bacteria (MDR/MDRO) among dogs and cats is considered an important component of the AMR problem, as carrier animals can maintain the circulation of resistant enterobacteria in households and veterinary facilities. Enterobacterales (in particular the *Enterobacteriaceae* family) are of particular importance in the structure of AMR in

companion animals due to their prevalence in both normal microbiota and among pathogens of various etiologies (skin and soft tissue infections, postoperative complications). At the same time, they are characterized by a high capacity for horizontal transfer of resistance genes, which contributes to the formation of MDR/MDRO profiles. At the molecular level, the spread of plasmid-associated determinants plays a key role, primarily extended-spectrum  $\beta$ -lactamases (ESBL) and AmpC (Formenti, 2021; Menezes, 2024).

A systematic review of risk factors for antimicrobial-resistant Enterobacterales in dogs highlights the importance of previous antibiotic use, hospitalisation, and certain clinical conditions that increase the likelihood of detecting resistant isolates (Karalliu, 2024). The emergence of carbapenemase-producing *Enterobacteriaceae* in small companion animals is a particular concern, as such resistance mechanisms are associated with antimicrobial drugs that are critical for treatment and may reflect intense selective pressure in the clinical setting. It has been noted that the link between inadequate infection control in veterinary clinics correlates with environmental contamination with carbapenemase-producing *Enterobacterales* and other MDR bacteria (Schmidt, 2020), and recent reviews and reports from various European countries confirm the growing importance of this problem in the treatment of small animals (Silva, 2022; Silva, 2024; Perreten, 2025).

Additional attention is drawn to reports of decreased sensitivity to colistin among isolates obtained from companion animals, particularly within the *Enterobacter cloacae* complex, which emphasizes the need for rational use of antimicrobial drugs and laboratory monitoring of treatment (Sato, 2022). According to a population study in China, MDR *Escherichia coli* was detected in 35.77% of companion animals (dogs — 40.96%, cats — 27.28%), which indicates a significant prevalence of resistant strains even outside the veterinary clinic (Teng, 2023). At the same time, the risk of colonization increases in clinical settings: in Switzerland, the proportion of MDRO-carrying animals was 15.5% on admission and 32.1% on discharge, and MDRO acquisition during treatment was recorded in 28.3% of cases, emphasizing the role of veterinary clinics as an environment for intensive selection and transmission of resistant microorganisms (Dazio, 2021b). Data from a hospital in Spain show a similar trend: the carriage of ESBL-producing *Enterobacteriaceae* in dogs increased from 25.0% on admission to 45.5% on discharge (Ortiz-Díez, 2023). Available data also confirm the possibility of MDRO transmission between humans and animals under conditions of close contact, which increases the significance of the problem within households and clinics (Dazio, 2021a; Hackmann, 2024; Menezes, 2024).

On this basis, the aim of this study is to summarize current data on the prevalence of MDR/MDRO *Enterobacterales* in companion animal practice and to identify key risk factors and areas for prevention, in particular through increased microbiological monitoring and infection control measures in veterinary hospitals (Karalliu, 2024; Monteiro, 2025).

**The aim of the study** was to assess the structure of clinically significant isolates of the *Enterobacteriaceae* family and the proportion of multidrug-resistant (MDR) strains in dogs and cats with clinical signs of infections of bacterial etiology in veterinary clinics in Kyiv in 2022–2025.

**Materials and methods.** The study was conducted in 2022–2025 in veterinary clinics in Kyiv, included dogs and cats with clinical signs of bacterial infections. Biological material was collected during routine diagnostic procedures after obtaining informed consent from the animal owners. A total of 63 clinical samples (n=63) were collected, including 17 fecal samples (n=17) and 46 samples from purulent-inflammatory wounds (n=46). The laboratory stage was carried out at the Department of Veterinary Epidemiology and Animal Health of the National University of Life and Environmental Sciences of Ukraine, using the material and technical resources of the Laboratory of Molecular Virology and Medical Microbiology with the Museum of Microorganisms Pathogenic to Humans of the State Institution “L.V. Gromashevsky Institute of Epidemiology and Infectious Diseases of the National Academy of Medical Sciences of Ukraine.”

Material from purulent-inflammatory wounds was collected with sterile swabs after preliminary cleaning of the wound surface with sterile saline solution (0.9% NaCl) to remove necrotic masses and surface exudate. The sampling was performed from the depth of the wound or from the area of active inflammation in order to obtain a clinically significant isolate. Fecal samples were collected in sterile containers in accordance with standard aseptic rules. The collected material was transported to the laboratory in thermal containers with cooling elements at a temperature of 2-8 °C, while maintaining the cold chain. Immediately after collection, swabs

with wound material were placed in Amies transport medium (AMIES), which ensures the viability of Gram-negative bacteria during transport.

The initial bacteriological examination included cultivation of clinical material on selective and differential diagnostic medium recommended for the isolation of enterobacteria. For this purpose, MacConkey agar was used to assess lactose fermentation and Endo medium to differentiate between lactose-positive and lactose-negative colonies. If necessary, meat peptone agar (MPA) was used to obtain isolated colonies. The cultures were incubated under aerobic conditions at 35–37 °C for 18–24 hours. After incubation, the growth and morphological characteristics of the colonies (size, shape, edges, surface, pigmentation) were evaluated, as well as the nature of growth on MacConkey agar. Biochemical identification of the isolated cultures was performed using standard tests that allow differentiation between members of the Enterobacteriaceae family. The results of the reactions were recorded after incubation at 35–37 °C in accordance with the manufacturer's instructions and generally accepted microbiological reference books.

The sensitivity of isolates to antimicrobial drugs was determined by the disc diffusion method (Kirby–Bauer) with interpretation of the results in accordance with EUCAST recommendations. The panel of tested drugs included  $\beta$ -lactams (ampicillin, amoxicillin/clavulanic acid), third-generation cephalosporins (cefotaxime, ceftriaxone, or ceftazidime), fluoroquinolones (enrofloxacin, marbofloxacin), aminoglycosides (gentamicin, amikacin), tetracycline, and cotrimoxazole. Phenotypic detection of extended-spectrum  $\beta$ -lactamases (ESBL) was performed using the double disc synergy test (DDST). Multidrug resistance (MDR) was defined as the insensitivity of an isolate to at least one antimicrobial agent in three or more classes of antimicrobial agents. The results of the study were summarized descriptively in the form of absolute (n) and relative (%) indicators, followed by presentation in tables.

**Results.** The data obtained indicate that in the conditions of veterinary practice in Kyiv in 2022–2025, *Escherichia coli* (44/63; 69.8%) dominated among the isolated representatives of the Enterobacteriaceae family, while the proportion of *Klebsiella* spp. was 19/63 (30.2%). This structure of isolates is expected for clinical material from small domestic animals, since *E. coli* is widely represented in the intestinal microbiota and is opportunistic (Formenti, 2021; Monteiro, 2025).

The detection of *Klebsiella* spp. as the second most frequent group is also clinically significant, as representatives of this genus are often associated with clinically associated infections and are capable of accumulating resistance factors, which complicates treatment (Menezes, 2024; Monteiro, 2025).

The multidrug resistance (MDR) rate of 15/63 (23.8%) reflects the presence of a significant proportion of isolates for which the choice of antimicrobial drugs is potentially narrowed. For veterinary practice, this means an increased risk of ineffective therapy and the need to focus on laboratory diagnostic results, and also highlights the importance of rational prescribing of antimicrobial agents at the clinic level (Karalliu, 2024; Monteiro, 2025). Given that the study covered material from animals with clinical signs of bacterial infection, the recorded proportion of MDR may reflect the cumulative effect of previous antibiotic use, duration, or recurrence of infection, and possible factors related to the animal's admission to the clinic (Dazio, 2021b; Ortiz-Díez, 2023).

An important factor in interpretation is the structure of the biomaterial: swabs from purulent-inflammatory wounds predominated in the sample (46/63; 73.0%), while fecal samples accounted for 17/63 (27.0%). Skin and soft tissue infections, especially chronic or complicated ones, are often accompanied by mixed infection, surface contamination, and the formation of microbial communities, which can support the persistence of pathogens and increase the likelihood of detecting strains with acquired resistance (Monteiro, 2025).

These findings are of practical significance in the context of the One Health concept, since companion animals can act as reservoirs and epidemiological “bridges” facilitating the circulation of resistant Enterobacteriales between households and veterinary clinics (Walther, 2023; Hackmann, 2024; Menezes, 2024). This reinforces the argument for systematic control in veterinary clinics: hand hygiene, routine disinfection of surfaces and instruments, separation of patient flows, proper handling pathogenic material (Schmidt, 2020; Dazio, 2021b; Monteiro, 2025).

The limitations of the study include the local nature of the sample, the moderate volume and uneven distribution of material types, which may affect the generalisation of results to other regions and other categories of patients. In addition, the lack of a detailed analysis of sensitivity profiles for each drug for all isolates limits the ability to detail practical recommendations for the choice of therapy; However, even under these conditions, the established proportion of MDR emphasizes the need for further studies with broader coverage and standardized data collection on antimicrobial susceptibility and resistance mechanisms (Karalliu, 2024; Menezes, 2024; Monteiro, 2025).

**Conclusions.** *Escherichia coli* dominated among the isolated enterobacteria — 44/63 (69.8%), while *Klebsiella* spp. accounted for 19/63 (30.2%), which characterizes the local microbiological profile of Enterobacteriaceae in clinical cases of bacterial infections in the city.

Multidrug resistance (MDR) was detected in 15/63 (23.8%) isolates, i.e., in almost every fourth case, which emphasizes the presence in practice of a significant proportion of strains with a potentially limited choice of antimicrobial agents. The recorded proportion of MDR indicates an increased risk of ineffective empirical antibiotic therapy and justifies the need for wider use of bacteriological testing and susceptibility testing for clinically significant cases, particularly in purulent-inflammatory lesions of the skin and soft tissues.

The results obtained indicate the importance of a systematic approach to combating AMR in veterinary institutions, which should include the rational use of antimicrobial drugs. Standardization of infection control measures (hand hygiene, disinfection, proper handling of wound material and excreta).

Further research should focus on expanding the sample (other cities/regions), balancing the structure of the material, and supplementing phenotypic screening (in particular ESBL) with molecular methods to clarify the mechanisms of resistance and assess possible routes of spread within the One Health approach.

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### Мультирезистентні ентеробактерії у ветеринарній практиці: сучасні виклики

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**Резюме.** Антимікробна резистентність у тварин-компаньйонів має зростаюче значення в межах підходу One Health через тісний контакт людей і домашніх тварин та ризик циркуляції мультирезистентних збудників у домогосподарствах і ветеринарних клініках. Метою дослідження було оцінити структуру клінічно значущих ізолятів родини Enterobacteriaceae та частку мультирезистентних (MDR) штамів у собак і котів із клінічними ознаками інфекцій бактеріальної етіології у ветеринарних клініках м. Київ у 2022–2025 рр. Дослідження виконано як одномоментне обсерваційне. Відібрано 63 клінічні зразки (n=63): фекалії — 17 (27,0%) та мазки з гнійно-запальних ран — 46 (73,0%). Зразки транспортували при +2...+8 °С; рановий матеріал відбирали стерильними тампонами з глибини/ділянки активного запалення та поміщали в транспортне середовище Amies (AMIES). Культивування проводили на агарі МакКонкі та середовищі Ендо з інкубацією 35–37 °С протягом 18–24 год; ідентифікацію здійснювали стандартними біохімічними тестами. Чутливість до антимікробних препаратів визначали методом Kirby–Bauer на агарі Мюллера–Гінтона з інтерпретацією за EUCAST; ESBL виявляли методом DDST. MDR визначали як нечутливість щонайменше до одного препарату в трьох або більше

класах. Дані узагальнювали описово (*n*, %). У результаті домінуючим ізолятом була *Escherichia coli* — 44/63 (69,8%), тоді як *Klebsiella spp.* становила 19/63 (30,2%). MDR виявлено у 15/63 ізолятів (23,8%), тобто майже кожен четвертий ізолят характеризувався мультирезистентністю.

У ветеринарних клініках м. Київ у 2023–2025 рр. серед клінічних ізолятів *Enterobacteriaceae* переважала *E. coli*, а частка MDR становила 23,8%. Результати підтверджують потребу в регулярному локальному мікробіологічному моніторингу, посиленні інфекційного контролю та раціональному застосуванні антимікробних препаратів у практиці лікування тварин-компаньйонів.

**Ключові слова:** антимікробна резистентність, мультирезистентність, *Enterobacteriaceae*, *Escherichia coli*, *Klebsiella spp.*, тварини-компаньйони, ветеринарні клініки, One Health.

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