ULST Timisoara
Multidisciplinary Conference on Sustainable Development
15-16 May 2025

Identification and Antimicrobial Resistance of potentially pathogenic bacterial species in Red foxes (*Vulpes vulpes*) from Western Romania

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Abstract: *Red foxes (Vulpes vulpes), as adaptable wild carnivores, play a significant role in the ecology of zoonotic pathogens due to their close proximity to human-influenced environments. The present study aimed to identify potentially pathogenic bacteria present in the intestinal flora of red foxes hunted in a hunting fund from Western Romania, offering insights into their potential role in environmental and public health. Rectal swabs were collected post-mortem from a cohort of red foxes and analyzed using classical microbiological techniques. Samples were cultured on selective and differential media designed to isolate Gram-negative bacterial species. Final bacterial identification was performed using the Vitek 2 compact system, allowing precise species-level classification. Furthermore, antimicrobial resistance was tested for all the identified strains using de Kirby-Bauer disk-diffusion method. The microbial flora was composed of species from the genera Escherichia, Proteus, Salmonella, Enterobacter, all of which include opportunistic or potentially pathogenic strains relevant to both veterinary and human medicine. Multi-drug resistance was also observed in more than half of the bacterial isolates. These findings suggest that red foxes may serve as reservoirs or vectors for antimicrobial resistant pathogenic bacteria, with implications for cross-species transmission, particularly in areas with overlapping habitats for wildlife, livestock, and humans. Continuous monitoring and microbiological surveillance of wild carnivores are recommended to better understand their role in the epidemiology of infectious diseases and to inform public health strategies.*

• Introduction

Although antimicrobial resistance (AMR) occurs naturally, its spread has accelerated due to widespread antibiotic use in human and animal health, as well as agriculture. The red fox (*Vulpes vulpes*), common across Europe and increasingly found in urban areas, can pick up resistant bacteria like MRSA, ESBL-producing *E. coli*, and *Salmonella* through scavenging in human waste. Because of this, foxes may serve as useful indicators of how AMR spreads in the environment.

This study set out to examine how often antibiotic-resistant gut bacteria appear in Romanian red foxes and to better understand their resistance patterns—an important step in applying the One Health approach to AMR surveillance in wildlife.

• Materials and methods

Rectal swabs were collected from 14 adult wild red foxes (*Vulpes vulpes*)—8 males and 6 females—sourced from the Buziaș hunting fund in Timiș County, western Romania. Foxes were obtained through regulated hunting or found deceased in the wild. Only intact adult carcasses were used to avoid age-related variation and contamination. Samples were collected within 24 hours postmortem or stored frozen to preserve bacterial integrity.

These foxes were part of Romania's national wildlife control program; none were killed specifically for this study, and ethical approval was not required.

Swabs were placed in Nutrient Broth or Buffered Peptone Water for non-selective enrichment, incubated at 37°C for 24 hours, and then streaked on selective media: TBX, XLD, EMB, and Rambach agar. Morphologically distinct colonies were Gram-stained and identified using the Vitek 2 Compact system with GN ID cards (bioMérieux, France).

Antibiotic susceptibility was assessed via Kirby-Bauer disk diffusion following CLSI and EUCAST standards. A total of 15 antibiotics across 7 antimicrobial classes were tested, includin the following: (I) Beta-lactams, represented by Penicillins (Ampicillin – AMP 10 µg, Amoxicillin – AML 30 µg), Cephalosporins (Ceftazidime – CAZ 10 µg, Cefoxitin – FOX 30 µg, Cephalexin – CL 30 µg), and Carbapenems (Imipenem – IMI 10 µg); (II) Aminoglycosides, including Gentamicin – CN 10 µg, Amikacin – AK 30 µg, and Tobramycin – TOB 10 µg; (III) Tetracyclines, represented by Tetracycline – TE 30 µg; (IV) Fluoroquinolones, including Ciprofloxacin – CIP 5 µg and Nalidixic Acid – NA 30 µg; (V) Sulfonamides, represented by the combination of Sulfamethoxazole-Trimethoprim – SXT 25 µg; (VI) Nitrofurans, represented by Nitrofurantoin – F 100 µg; and (VII) Amphenicols, represented by Chloramphenicol – C 30 µg. Results were interpreted as Susceptible, Intermediate, or Resistant. *Escherichia coli* ATCC 25922 was used as a quality control strain.

• Results and discussions

Bacteriological analysis confirmed that all 14 rectal swab samples collected from red foxes were positive for cultivable bacteria, with visible colony growth observed on at least one of the selective media. TBX agar showed the highest isolation rate, with bacterial growth in all samples (100%), followed by XLD agar (86%, 12/14), EMB agar (72%, 10/14), and Rambach Agar (29%, 4/14). Gram staining confirmed that all isolates were Gram-negative bacteria (GNB).

A total of 12 representative isolates were selected for species-level identification using the Vitek 2 Compact system. The most frequently identified species was *Escherichia coli*, present in 8 of the 12 isolates (67%), followed by *Proteus mirabilis* in 2 samples (17%), and *Salmonella* spp. and *Enterobacter* spp. each in one sample (8%). The *Salmonella* spp. isolate was further confirmed as *Salmonella enterica* subsp. *enterica* by molecular analysis.

Antimicrobial susceptibility testing was performed on all 12 isolates against 15 antibiotics. The highest resistance rates were recorded for Ceftazidime (CAZ – 92%), followed by Ciprofloxacin (CIP – 58%) and Amoxicillin (AML – 58%). Moderate resistance was observed for Ampicillin (AMP), Imipenem (IMI), Gentamicin (CN), Amikacin (AK), and Nalidixic Acid (NA), each at 50%. In contrast, the highest susceptibility rates were found for Chloramphenicol (C – 92%), followed by Nitrofurantoin (F – 83%), Cephalexin (CL – 83%), Sulfamethoxazole-Trimethoprim (SXT – 75%), and both Cefoxitin (FOX) and Tobramycin (TOB) at 67%.

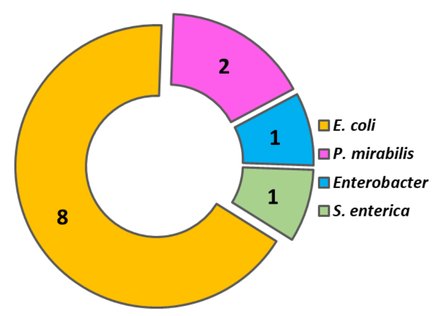

Figure 2. Gram-negative bacterial species tested by the diffusimetric method

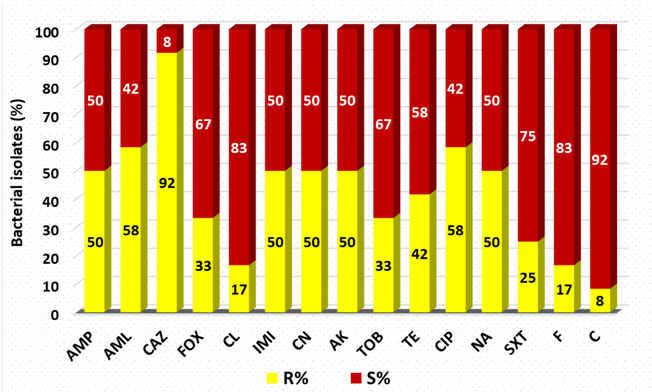

Figure 2. Antibiotic resistance profile of the tested bacterial species

All eight *E. coli* isolates showed resistance to most beta-lactams, especially CAZ, AMP, and AML. Resistance was also noted for aminoglycosides (CN, AK) and fluoroquinolones (CIP, NA). However, susceptibility was generally retained for FOX, CL, TOB, TE, SXT, C, and fully for F. The two *P. mirabilis* isolates showed variable resistance: one was resistant to 11 antibiotics, while the other to only 3. Both remained susceptible to AMP, AML, SXT, and C. The *S. enterica* isolate was resistant to 10 of the 15 antibiotics tested but susceptible to CL, AK, F, and C. The *Enterobacter* spp. isolate showed susceptibility to most antibiotics, with resistance noted only for AML, CAZ, and CIP.

Table 3. Antimicrobial resistance profile of the identified species

Crt. No.	Tested Strains (n)	Antimicrobial Resistance Profile
1	*Escherichia coli* (8)	*AML, CAZ, FOX, IMI, CN, AK, TOB, CIP, NA;
2		*AMP, AML, CAZ, FOX, CL, IMI, CN, AK, TOB, TE, CIP, NA, SXT;
3		*AMP, AML, CAZ, IMI, CN, AK, NA, SXT;
4		*AMP, CAZ, AK, CIP;
5		AMP, AML, CAZ, CN;
6		AMP, AML, CAZ, NA;
7		*CAZ, AK, TE, C;
8		CAZ, CIP;
9	*Enterobacter spp.* (1)	AML, CAZ, CIP;
10	*Proteus mirabilis* (2)	*CAZ, FOX, CL, IMI, CN, AK, TOB, TE, CIP, NA;
11		*IMI, TE, F;
12	*Salmonella enterica* (1)	*AMP, AML, CAZ, FOX, IMI, CN, TOB, TE, CIP, NA, SXT;

Multidrug resistance (MDR), defined as resistance to at least one agent in three, or more antimicrobial classes, was observed in 67% of isolates. More precisely, multidrug resistance was observed in five out of eight *E. coli* isolates, both *P. mirabilis* isolates, and the *S. enterica* isolate.