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A study on Antimicrobial Resistance of Bacteria isolated in Wildlife in Western Romania

Moza A.C.[1], Bucur* I.M.[1], Nichita I.[1], Gros R.V.[1], Plotuna A-M.[1], Mederle N.[1], Marin A.[1], Bulacu A.[2], Tîrziu E.[1]

[1]University of Life Sciences "King Michael I" from Timişoara, Calea Aradului 119, 300645 Timişoara, Romania
[2]Romanian Wilderness Society, 335500, Carpați 5, Hațeg, Romania
**Corresponding author: iulia_bucur@ymail.com*

Abstract: *Antimicrobial resistance (AMR) is considered by the World Health Organization (WHO), as one of the greatest public health risks of this century. The aim of the study was to identify and determine the presence of antibiotic-resistant potential pathogens in different species of wild mammals (n=25), in Romania, represented by Vulpes vulpes (Red fox), Canis aureus (Golden jackal), Capreolus capreolus (Roe deer), Dama dama (European fallow deer), and Felis silvestris (Wild cat). Individuals were sampled by rectal swabbing and the isolates were obtained by cultivation on the following agars: TBX, Oxoid Chromogenic Listeria, XLD, and Chapman. Bacterial strains (n=29) were identificatied with Vitek 2 Compact system, and susceptibility to antimicrobial substances was determined through, both, Vitek 2 and disk diffusion method. The isolation frequencies were as follows: Escherichia coli (48.27%), Staphylococcus spp. (41.37%) comprised by seven species, Proteus (6.89%) with two species, and Salmonella spp. (3.44%). Only one strain of E. coli, isolated from the roe deer, was resistant cu ampicillin. One isolate of Staphylococcus pseudintermedius originated from the golden jackal was multi-drug resistant. Overall, 37.93% of the identified strains presented resistance to at least one of the antibiotics, with variable resistance towards one, two, respectively three antimicrobials.*

• Introduction

Antimicrobial resistance (AMR) in bacteria is considered a worldwide issue in, both human and veterinary medicine, due to its zoonotic characteristic. Wildlife is essential for sustaining ecological balance and biodiversity, and the link between the humans, animals, and the environment was accepted by The One Health, raising awareness of the AMR bacteria hazard Over the last ten years, significant worry has emerged regarding the rise in bacterial resistance to antimicrobials within wild animal populations, including pathogens such as Escherichia coli, Salmonella spp., Stahylococcus spp., Listeria monocytogenes. Despite the fact that antimicrobial resistance represents a global health risk, a lack in studies on wildlife AMR bacteria can be observed. In this regard, the aim of this study was to determine the prevalence and antimicrobial resistance pattern in potentially pathogenic gut bacteria that were isolated from different wildlife species in Romania.

• Material and method

Rectal swabbing was performed with cotton swabs on 25 wild animal carcasses coming from several counties in Romania, respectively Arad, Timiș, Bihor and Hunedoara. The carcasses originated from fresh road-kills, hunted animals, or individuals that were found dead in the natural environment, and the harvested wildlife species were Roe deer (*Capreolus capreolus*, n=8), Fallow deer (*Dama dama*, n=3), Red fox (*Vulpes vulpes*, n=5), Golden jackal (*Canis aureus*, n=3), Wild cat (*Felis silvestris*, n=4), and European badger (*Meles meles*, n=2).

After a pre-enrichment in Nutrient broth or Buffered peptone water, samples were transferred onto selective and differential culture media to facilitate the growth of different species (TBX and XLD Agar for Gram-negative bacteria; Manitol Salt Agar (MSA) and Chromogenic Listeria Agar (CLA) for Gram-positive bacteria). specific colonies were examined microscopically through smear preparations followed by Gram staining. Lastly, the selected isolates were identified with the Vitek 2 Compact system . For this, identification cards were used for both Gram-negative and Gram-positive bacteria. The antibiotic susceptibility was determined initially by Kirby-Bauer disk diffusion (DD) test using the following discs: ampicillin (AMP; 10 µg), amoxicillin/clavulanic acid (AMC, 30 µg) streptomycin (S, 300 µg), gentamicin (GN; 120 µg), cefalexin (CN 30 µg), chloramphenicol (CL; 30 µg), erythromycin (E; 15 µg), tetracycline (TE; 30 µg). Additionally, isolates that presented resistance to at least one of the tested antimicrobials were further examined with Vitek 2 Compact system.

• Results and discussions

The bacteriological examinations showed that all 25 samples were positive to cultivable bacteria, colonies being present on at least one of the selective culture media.

Subsequent to the cultural and microscopical examinations of the isolates, a total of 29 bacterial isolates had been selected to be identified using the advanced Vitek 2 Compact system.

The results showed that the highest prevalent bacterial species was *Escherichia coli* (14/29, 48.27%), followed by *Staphylococcus* spp. (12/29, 41.37%) comprised by seven species, *Proteus* (2/29, 6.89%) with two species (*P. vulgaris and P. mirabilis*), and *Salmonella* spp. (1/29, 3.44%).

The results obtained after the antibiotic susceptibility assays showed that the identified strains presented several patterns of resistance (Table 1). Regarding cervids, in roe deer a strain of E. coli and one of *S. vitulinus* showed resistance towards ampicillin, respectively erythromycin and tilmicosin. Furthermore, *S. warneri* isolated from the fallow deer presented resistance towards tetracycline.

The most antimicrobial resistance was observed in the strains isolated from the wild carnivores. Regarding the red fox the antimicrobial resistance pattern of the tested strains were as follows: *Salmonella* spp. (cephalexin, amikacin and gentamicin), *Proteus vulgaris* (ampicillin, cephalexin), *S. lentus* (erythromycin, tetracycline), and *S. vitulinus* (tetracycline, gentamycin). Moreover, one strain of *S. pseudintermedius* isolated from the golden jackal had resistance to erythromycin, enrofloxacin and clindamycin. The *Proteus mirabilis* isolate from the badger expressed resistance towards ampicillin and tetracycline and, lastly, one strain of *S. vitulinus* identified in the wild cat was resistant to gentamycin.

Overall, 37.93% (11/29) of the identified strains presented resistance to at least one of the tested antibiotics, out of which 3, 6 and 2 isolates showed resistance towards one, two, respectively three antimicrobials. Additionally, the *Staphylococcus pseudintermedius* isolated from the golden jackal manifested multi-drug resistance (MDR) being resistant to one antimicrobial from each of the following three classes of antibiotics: macrolides (erythromycin), lincosamides (clindamycin) and quinolones (enrofloxacin).

The results obtained in the research are consistent to other authors' findings, however in some wild species, we could not compare the results due to the lack of current research.

Table 1. Antimicrobial resistance profile of the identified species

Crt. No.	Animal species	Tested Bacteria (n)	AMR profile
1	Roe deer	*E. coli*	AMP
		S. vitulinus	E, TIL
2	Fallow deer	*S. warneri*	TE
3	Red fox	*Salmonella spp.*	CN, AMK, GN
		Proteus vulgaris	AMP, CN
		S. lentus	E, TE
		S. vitulinus (2)	TE, GN
4	Golden jackal	*S. pseudintermedius*	E, ENR, CM*
5	Wild cat	*S. vitulinus*	GN
6	Badger	*Proteus mirabilis*	AMP, TE

AMK – Amikacin; AMP – Ampicillin; CN – Cephalexin; CM – Clindamycin; E – Erythromycin; ENR – Enrofloxacin; GN – Gentamycin; TE – Tetracycline; TIL – Tilmicosin; * - Multi-drug resistance.

• Conclusions

A total of twenty-nine isolates were identified from six wildlife species with Vitek 2 Compact system, and were included in four bacterial genera.

The antimicrobial resistance was determined by both disc-diffusion test and Vitek 2 system, recording resistance to at least one of the tested antibiotics in 37.93% (11/29) of the identified strains.

One strain of *Staphylococcus pseudintermedius* from the golden jackal showed multi-drug resistance (MDR) being resistant to erythromycin, enrofloxacin and clindamycin.

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