Summary

Escherichia coli (*E. coli*) is a natural component of the human and animal microbiota and is ubiquitous in the environment of humans and animals. Some strains have also developed many traits and adaptations to cause intestinal or extraintestinal infections in the host. In domestic and foreign literature, there are many studies on the resistance of *E. coli* bacteria to commonly used antimicrobial agents. A group of livestock animals that is best known for resistance is the group of animals subject to obligatory resistance monitoring. Several research papers have been published in Poland on the resistance of *E. coli* isolated from companion animals, which can easily transfer drug-resistant bacteria to humans through close contact.

Free-living animals seem to be another particularly interesting research group. Since they do not undergo targeted therapy, free-living animals should by definition be carriers of a lower percentage of drug-resistant strains and show a realistic representation of the level of drug resistance in the environment. On the other hand, in the modern environment, there are many animal species with high synanthropization potential. Such species as the red fox, beech marten, or mink increasingly often approach farms and human households to find easily accessible sources of food. In this way, these animals can contribute to the transfer of drug-resistant microorganisms to other groups of animals, humans, and the environment. In Poland, data on the occurrence of antimicrobial resistance in *E. coli* isolated from free-living animals are so far limited. This is probably related mainly to the difficulties in accessing research material.

This work is a multi-level analysis of drug resistance in *Escherichia coli* strains isolated from free-living animals. In carrying out the research, individual species of animals were analyzed in terms of the predominant type of diet, which, depending on the level of differentiation, may affect the level of drug resistance of the tested strains. The level of drug resistance of strains from carnivores, omnivores, and herbivores was tested separately. A separate research group were isolates with the phenotype of resistance to third generation cephalosporins (cephalosporin resistant CR). The study was conducted with a unique approach to the isolation stage of strains using combinations of four media and increasing the probability of detection of several strains resistant to at least one drug from a single trial. Strain differentiation was carried out at the phenotypic level by examining the resistance of the strains and at the genotypic level by analyzing the genomic profiles of the obtained isolates. The occurrence of genetic determinants of resistance and a wide panel of genes related to virulence were analyzed using molecular

The use of several media for isolation of the strains yielded strains from 71.6% of samples from carnivores and from 50.9% of samples from omnivores and herbivores resistant to at least one antibacterial substance. The isolated strains showed great diversity in terms of both the resistance phenotype and genomic profiles. The initial application of the disc diffusion method to exclude the same isolates from a given sample (risk of overestimation of the results) allowed elimination of only 13 strains. In turn, the genomic profiles of isolates obtained using the ADSRRS-fingerprinting method differed in terms of both the number of bands (from 5 to 19) and the size of the fragments obtained.

The tested *E. coli* strains showed leading resistance to ampicillin (98.3%), tetracycline (69.7%), and sulfamethoxazole (52.6%). In all research groups, a similar proportion of multi-drug-resistant strains (resistance to at least three antibacterial substances from different groups) was obtained, amounting to approximately 70%. Resistance to ciprofloxacin was demonstrated in 24% of isolates; however, the highest share was noted in strains resistant to cephalosporins. Phenotypic resistance has been confirmed by the presence of genetic determinants of resistance mainly of the *tetA* (in tetracycline-resistant strains) and *sul2* (in sulfamethoxazole-resistant strains) genes. The most common genes determining plasmid-mediated quinolone resistance (PMQR) resistance were *qnrS*, *qnrB*, and *aac(6')-Ib*. Cephalosporin-resistant strains were characterized by the production of a diverse panel of acquired broad-spectrum beta lactamases (ESBL) and cephalosporinases (AmpC). This phenotype was most often associated with the expression of the bla_{CMY-2} genes as well as the $bla_{CTX-M-1}$ and $bla_{CTX-M-9}$ genes.

The analysis of virulence genes revealed the most common presence of strains causing extraintestinal infections (ExPEC) and single strains with the ETEC (enterotoxicogenic *E. coli*) and EHEC (enterohemorrhagic *E. coli*) pathotype. The most common sequence types among the studied *E. coli* strains were those belonging to the clonal complexes: 10, 155, 23, and 69.

The obtained results confirm the necessity to study free-living animals as a potential reservoir of *E. coli* strains resistant to commonly used antimicrobial substances. The investigations have shown a positive relationship between cephalosporin resistance and the presence of PMQR genes. There was also no correlation between the presence of the multi-drug-resistant and virulent phenotype among the *E. coli* isolates studied.

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