ECOLOGICAL ROLE OF BACTERIAL ISOLATES IN PROTECTED WILD BIRDS

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Summary: All wild animals require continuous monitoring carried out by human and veterinary medicine, because their characteristics can be an indicator of interaction between the intact ecosystems and regulated,(controlled) part of the ecosystems that are actively managed by humans. Some characteristics of wild animals that may have importance for human ecosystem and nature are related to bacterial species which inhabits the game, and control of bacterial susceptibility to antimicrobial drugs. For these reasons, the subject of our study was to investigate and analyse sensitivity of bacterial strains isolated from the samples collected from wild animals to antibiotics. The aim of this paper was to determine whether the resistance to tested antibiotics occurred in some of the isolated bacteria..Several different bacterial species were isolated. The researches indicate that certain bacterial species isolated from the samples originating from wild animals show resistance to the tested antimicrobials.

Keywords: wild animals, bacteria, resistance, ecosystem

Introduction

The connections between a man and the nature are manifold. Human interest in nature has economic, social, biogeography, as well health, scientific and environmental aspects. Wild animals constitute one of the most important part of the nature. For these reasons, the legislation is upgrading continuously in order to achieve a comprehensive protection of wildlife. By adopting Act on Ratification of the Convention on the conservation of European wild flora and fauna and natural habitats (Bern Convention), in November 2007, the Republic of Serbia committed to apply all the rules laid down by the Convention. Two levels of wild animal protection are distinguished: strictly protected and protected species, while the Convention does not recognize the category "game out of protection regime." Is it sufficient to protect wild game or is it necessary to view a threat to wild game in terms of interaction between a man and the nature? Some of the substances that are discharged into the nature can contribute to the change in the environment and adaptation of wild animals, even when there is time shortage [1]. Antibiotics are one of the widespread substances, but under a strict control, that come into nature, directly or indirectly, and requires adaptation by the animals. Despite efforts in protecting the nature from adverse effects of antimicrobial drugs, they are clearly left their mark on the bacterial flora in animals [5]. The possibility of occurrence and persistence of antimicrobial resistance in nature [13], is a danger not only for members of the ecosystem where it occurs, but for the humans, since in a cycle: hunt, surface water, tourism in nature, it returns to man in time and manner when it is least expected. Thus, the subject of our study was to investigate and analyze the sensitivity of bacterial strains isolated from samples collected from wild birds to antibiotics. The aim of this paper is to determine whether in some of the isolated bacteria appeared resistance to some of tested antibiotics. We have an opinion that data obtained can be useful in suggesting that nature and ecosystem of wild game is threatened by the proliferation of antibiotics.

Material and methods

The examined material originated from strictly protected species of wild birds (5 from an eagle, 2 from storks, 2 from herons, 3 from ducks, 6 from swans). The laboratory samples were taken from the birds found dead within hunting grounds around water bodies (swamps or rivers). Parenchymatous organs: liver, heart, spleen, and intestinal parts were taken after careful opening of the carcass in a manner aimed to avoid body contamination. .

The samples for testing were placed by direct streaking on nutritive media (blood agar) and selective media (differential salmonella agar), previously sterilized by a burner. Anaerobic bacteria were tested by streaking on thioglycolate broth that was thermically treated in a water bath (80oC/10min) in order to neutralize any contaminants [10]. The content of intestine was streaked on buffered peptone water at 37oC for 24 h, after which we transferred to 100 µl semi solid Rappaport Vassiliadis Medium (MSRV) medium and incubated at 41.5o C for 24-48h. The colonies that appeared as Salmonella species were transferred to XLD and to Salmonella differential agar, and the identification of sera for agglutination [12] was carried out.

The sensitivity of isolated strains was tested according to the Permian CLSI recommendations (2006) [2]. We used antibiotic disks Tetracycline (30 µg), Streptomycin (10 µg), Trimethoprim + Sulphamethoxazol (25 µg

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(Trimethoprim1.25µg + Sulphamethoxazol 23,75 mg)), Lincomycin (2 µg), Enrofloxacin (5 µg), Neomicin (30 µg), floron (30 µg), Flumequin (30 µg), Gentamycin (10 µg), Colistin (10 µg), Erythromycin (15 µg), Amoxicillin (25 µg), Doxycycline (30 µg), Nalidixic acid (30 µg), Ampicillin (10 µg) produced by Bioanalyse and Linco-spectine (109 µg (Lincomycin 9µg +Spectinomicin100 µg)) manufactured by Oxoid.

Results and Discussion

The number of samples in our study was limited because organs were taken from the strictly protected animals, hunting on which is not permitted. Among analyzed samples, 5 originated from an eagle, 2 from storks, 2 from herons, 3 from ducks and 6 from swans. The following bacterial species were detected in the samples: *Escherichia coli, Salmonella enteritidis, Clostridium perfringens, Bacillus* sp*., Aeromonas* sp*., Staphylococcus* sp., *Micrococcus* sp. *Proteus vulgaris, Streptococcus beta hem., Streptococcus alpha hem*.

In Table 1 the results of susceptibility testing of isolated bacteria to antibiotics are displayed. Data are presented according to bacterial species, rather than animal species from which they were isolated. The values given for data are an average value for isolates that were repetitive, or for those bacterial strains that were isolated in different animals.

In the present study the isolated bacterial flora (*Salmonella* and *Clostridium* species) were similar to the results of other researchers [3]. Certainly, in the literature data some of the most important bacterial species, such as *Salmonella*, have been researched where bacteriological, but also parasitological, role is mentioned [6], [11]. Their role becomes important in case of migratory birds that can carry these pathogens form one continent to another. The importance of migratory birds and the role they have as a group of researchers in Belgium [7] has researched a reservoir of resistant strains. In our studies, we presented data on the presence of other bacterial species, belonging to a group of saprophytic or conditionally pathogenic bacteria, because we aimed to determine their susceptibility to antimicrobial drugs.

Table 1. Susceptibility of bacterial isolates to antibiotics

	E. coli	*Salmonella*	*Aeromonas*	*Proteus*	*Clostidium*	*Bacillus*	*Staphylo. sp.*	*Micrococcus*	*Sc. beta hem.*	*Sc. alfa hem.*
1. Tetracycline	S	S	S	S	S	S	S	S	S	S
2. Streptomyicin	R	I	I	R	R	I	S	S	R	R
3. Neomicin	R	I	I	I	R	S	I	I	R	I
4. Floron	I	I	I	I	I	S	S	S	S	S
5. Flumequin	S	S	S	S	S	S	S	S	S	S
6. Gentamycin	R	R	S	I	I	I	S	S	R	I
7. Linco-spectyn	R	R	R	I	S	S	S	S	R	I
8. Enrofloxacin	S	S	S	I	R	S	S	S	S	S
9. Colistin	R	R	I	I	R	I	I	I	R	R
10. Doxycycline	R	R	S	I	S	S	S	S	I	I
11. Amoxicillin	I	S	R	I	S	S	S	S	S	S
12. Nalidixic acid	S	S	R	R	R	S	S	S	S	S
13. Erythromycin	R	R	S	I	S	I	S	S	R	I
14. Ampicillin	I	I	R	I	S	S	S	S	S	S
15. Lincomycin	R	R	R	R	S	I	I	I	R	R
16. Trimethoprim+sylapham.	S	S	S	S	R	S	S	S	S	S

(R-resistance, I-intermediate, S-sensitive)

The examination of the resistance of *E. coli* isolated from materials originating from wild birds that live near water bodies [9] in Poland showed that about one-third of isolated strains carry resistance to some of the tested antibiotics (cefotaxime, ciprofloxacin and nalidixic acid). In our study, the bacteria from Gram-negative group are very or partially resistant to the group of aminoglycosides (streptomycin, neomycin, gentamycin), macrolides (lincomycin, erythromycin), linco-spectyn and colistin. *E. coli* and *Salmonella* showed sensitivity to drugs from the group of flour quinolones, and to tetracycline and trimethoprim + sylpametaksazol, and the sensitivity shown by these isolates and other Gram-negative organisms. Susceptibility of isolated *Enterococcus sp*. samples of wastewater from livestock farms and samples from wild animals [8] shown that antimicrobial resistance spread beyond the farm

and enters into the nature. This confirms the fact that use of drugs in veterinary medicine man can be difficult for keeping only in a specific range.

The researchers from Italy [4] studied the importance of studying migratory birds and their ability to be an important link in the spreading chain of pathogenic microorganisms. The results indicate that bacteria from the family *Enteribacteriacae* showed a high percentage of susceptibility to sulphamethoxazole / trimethoprime, cefotaxime, nalidixic acid, chloramphenicol and tetracycline, but on the other hand, almost 50% of the isolates showed the resistance to ampicillin, amoxicillin-clavulanic acid, and streptomycin. In our testing, the isolates showed sensitivity to tetracycline and trimethoprim + sylphametaksazol and flour quinolone products, while the resistance was present in the drugs from the group of aminoglycosides and macrolides.

Conclusion

The control of wild birds on the presence and prevalence of some bacterial species is important not only as a source to obtain epizootiological data, but as a possibility to control the pathogens. They are important for the prevention of infectious diseases that may threaten the health of domestic and wild animals, including humans, but also in terms of controlling the presence of bacterial strains that may carry resistance genes. These bacteria spread very fast and widely, and the possibility of unwished transfers of their negative characteristics is possible which can threaten the use of antibiotics when it is necessary. This study draws attention to the fact that controlled use of drugs has no alternative, and the care about the use of antibiotics must to be constantly improved

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