Susceptibility of *Enterobacteriaceae* from the Alpine Accentor *Prunella collaris*

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Abstract

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Occurrence of bacterial species of the family *Enterobacteriaceace* and their susceptibility and minimum inhibitory concentrations (MIC_{90}) to 13 selected antimicrobial agents was studied in 22 faecal samples of the alpine accentor *Prunella collaris.* Alpine accentor is our main research avian model for the studying of the interaction between the comensal microflora and enviroment. We isolated *E. coli, Serratia marscescens, Hafnia alvei, Citrobacter* spp. and *Morganella morgani* strains. We found no resistance of *E. coli* and *Serratia* spp. The strains *Serratia marcescens* and *Morganella morgani* were 100% resistant to ampicilin, ampicilin+sulbactam and cefuroxim. *Hafnia alvei* showed resistance only to cefuroxim and *Citrobacter* spp. only to ampicilin. The results of our study have shown that in high altitude mountains – despite of deterioration of the environment – no selection pressure exists as yet of using antimicrobial agents and only intrinsic resistance of *Enterobacteriaceae* strains occurred to investigated antibiotics. This fact was documented by comparing the susceptibility of *E. coli* strains isolated from faecal samples of *Prunella collaris* with susceptibility of *E. coli* strains acquired from 218 patients from Central Military Hospital in 2001.

Susceptibility of *Enterobacteriaceae, Prunella collaris,* antibiotic resistance

An inevitable side effect of the use of antibiotics is the emergence and dissemination of resistant bacteria. Most retrospective and prospective studies show that after the introduction of an antibiotic not only the level of resistance of pathogenic bacteria, but also of commensal bacteria increases. Commensal bacteria play an important role in protection from enteric infections (Kmeť et al. 1993), however, constitute a reservoir of resistance genes to (potentially) pathogenic bacteria, also. Their level of resistance is considered to be a good indicator for selection pressure by antibiotic use and for resistance problems to be expected in pathogens (Murray 1992). Monitoring the prevalence of resistance in indicator bacteria such as faecal *Escherichia coli* and enterococci in different populations of animals, patients and healthy humans, makes it feasible to compare the prevalence of resistance and to detect the transfer of resistant bacteria or resistance genes from animals to humans and vice versa (Van den Bogaard 2000).

The Alpine accentors were studied in the three geomorphologic areas: high altitude chains of the High and Low Tatra mountains, and the wintering site Malino Brdo, Great Fatra mountains. The summer study regions lie above the timber and dwarf pine line usually between 1 800 and 2 650 m above sea level. The habitats were dominated by alpine meadows and by rocky parts (Drgonová and Janiga 1989; Nakamura and Ueuma 1996).

Only few interactions exist between the alpine accentor and humans because this is a highly discrete bird whose sites of reproduction are far from any human influence. Perhaps only the development of ski resorts made it possible for this species to develop the wintering at altitude while giving access to him an easy food.

The aim of the study was to study the susceptibility of *Enterobacteriaceae* isolated from

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the alpine accentor *Prunella collaris*, to compare resistance of *Enterobacteriaceae* from alpine accentors with resistance of *Enterobacteriacea*e from clinical samples of hospitalized patients in Central Military Hospital in 2001 and to evaluate the influence of environment to susceptibility of *Enterobacteriaceae.*

Materials and Methods

Gram-negative strains of *Enterobacteriaceae* were isolated from 22 faecal samples of *Prunella collaris* and 218 strains *E. coli* were isolated from 218 patients (urinary tract infections -57 %, wounds - 25%, surgical samples- 7%, others -11%).

Specimens of droppings were collected in sterile plastic containers and sent to the laboratory where they were inoculated on non-selective (Columbia blood agar, Oxoid) and selective medium (Endo agar, OXOID). All plates were incubated at 37 °C.

Isolates were identified to species (genus) level using standard laboratory methods for routine, and exact definitive identification of strains of family *Enterobacteriaceae* was made using ENTEROtest 24 (PLIVA - Lachema). Configuration of tests enables a highly reliable identification without using other additional tests.

The susceptibility (MIC_{90}) of important strains of *Enterobacteriaceae* was determined by the MIDITECH automated colorimetric MIC reading for antimicrobial susceptibility testing (Gattringer et al. 2002). This system is a modification of the standard broth microdilution method that uses a 3-(4,5-dimetylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) dye for detecting viable bacteria.

Results and Discussion

In our study faecal samples were examined of *Prunella collaris,* a mountain bird living in the high altitude mountain environment. We obtained 34 strains of family *Enterobacteriaceae.* Total bacterial species are presented in Fig.1.

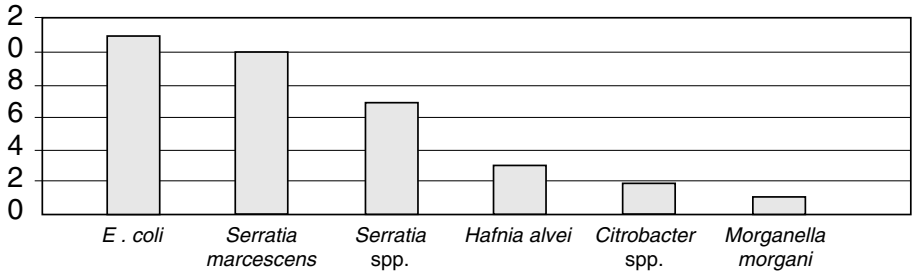

Fig. 1. Prevalence of *Enterobacteriaceae* strains isolated from 22 faecal samples of *Prunella collaris*

We found no resistance of *E. coli* and *Serratia* spp. The strains *Serratia marcescens* and *Morganella morgani* were 100% resistant to ampicilin, ampicilin+sulbactam and cefuroxim. *Hafnia alvei* showed resistance only to cefuroxim and *Citrobacter* spp. only to ampicilin.

The presented results are in good agreement with the intrinsic resistance of *Enterobacteriaceae.*

Susceptibility of *E. coli* strains isolated from faecal samples of *Prunella collaris* compared with that of *E. coli* strains of Central Military Hospital 218 patients in 2001 was excellent in all *Prunella* samples. It can be seen that the selection pressure of using antimicrobial agents changes the resistance. *E. coli* isolated from clinical samples of 218 patients had 42% resistance to ampicillin (92 strains), 24% resistance to piperacilin (51 strains), 22% resistance to COT (48 strains), 16% to ciprinol (35 strains) , 10% to cefotaxim (21 strains) and less than 5% resistance to other seven antimicrobials (Fig. 2). The presented results are in agreement with resistance among 590 clinical *E. coli* isolates (Kmeťová et al. 2001). These autors observed that the highest resistance of *E. coli* was to ampicillin (46%.)

Table 1
Resistance of *Enterobacteriaceae* strains isolated from faecal samples of *Prunella collaris* to selected antimicrobial agents in per cent

	E. coli (11)[a]	*Serratia marcescens* (10)	*Serratia* spp. (7)	*Hafnia alvei* (3)	*Citrobacter* spp. (2)	*Morganella morgani* (1)
AMP	0b	100	0	0	100	100
A+IB	0	100	0	0	0	100
PIP	0	0	0	0	0	0
P+IB	0	0	0	0	0	0
CXM	0	100	0	100	0	100
CTX	0	0	0	0	0	0
CAZ	0	0	0	0	0	0
CPO	0	0	0	0	0	0
CPM	0	0	0	0	0	0
GEN	0	0	0	0	0	0
AMI	0	0	0	0	0	0
CIP	0	0	0	0	0	0
COT	0	0	0	0	0	0

a - total number of isolates
b - value of resistance in %

and the lowest resistance was found against two third generation cephalosporins, ceftazidime (0.68%).

The normal bacterial flora in birds develops an effective defense mechanism against infection and illness. It is during times of illness, injury and excessive stress that the normal microflora, pH and digestive process can be effected. Examples of stress that can alter a birds physiological microflora populations in the gut are: feed or formula changes, poor nutrition, hatching, weaning, inadequate or close housing, shipping, excessive temperatures, exposure to pathogenic microorganisms or viruses, weakened immune system, and over exertion (McFarland 2000; Kleessen et al. 2000; Alverdy et al. 1998).

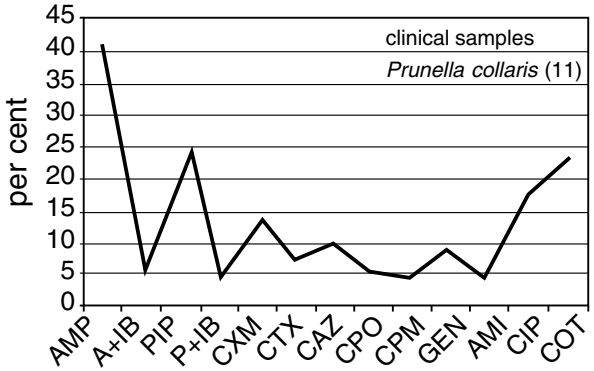

Fig. 2. Comparison of *E. coli* resistance to selected antimicrobial agents
AMP – ampicilin, A+IB – ampicilin + sulbactam, PIP – piperacilin, CXM – cefuroxim, CTX – cefotaxim, CAZ – ceftazidim, CPO – cefpirom, CPM – meropenem, GEN – gentamycin, AMI – amikacin, CIP – ciprinol, COT – trimetoprim + sulfonamid

Prunella collaris is our main research avian model for the study of interactions between the comm;ensal microflora and enviroment. The results of our study confirm that in high altitude mountain environment – despite of deterioration of the environment – no selection pressure exists of using antimicrobial agents and only intrinsic resistance of *Enterobacteriaceae* strains occurred to investigated antibiotics.

Citlivosť enterobakteriaceí u vrchárky červenkastej (*Prunella collaris*)

Študovali sme výskyt bakteriálnych druhov čeľade *Enterobacteriaceae* a ich citlivost a minimálnu inhibičnú koncentráciu na 13 vybraných antimikrobiálnych látok v 22 vzorkách trusu vrchárky červenkastej. Tento vysokohorský spevavec je našim hlavným vtáčím modelom v štúdiu vzťahu medzi komenzálnou mikroflórou a životným prostredím. Izolovali sme nasledovné druhy baktérií: *E. coli, Serratia marscescens, Hafnia alvei, Citrobacter* spp. a *Morganella morgani.* Kmene *E. coli* a *Serratia* spp. boli citlivé na antibiotiká a kmene *Serratia marcescens* a *Morganella morgani* boli rezistentné na ampicilin, ampicilín+sulbaktám a cefuroxim. *Hafnia alvei* bola rezistnentná iba na cefuroxim a *Citrobacter* spp. len na ampicilín. Výsledky nášho štúdia ukázali, že vo vysokohorskom prostredí, napriek znečisteniu životného prostredia, neexistuje selekčný tlak antibiotík a preukázaná rezistencia u *Enterobacteriaceae* je iba prirodzenou rezistenciou na sledované antibiotiká. Tento fakt sme dokumentovali porovnaním citlivosti *E. coli* zo vzoriek *Prunella collaris* s citlivosťou 218 kmeňov *E. coli* získaných z klinických vzoriek 218 pacientov ÚVN Ružomberok v roku 2001.

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