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Prevalence of Antibiotic Resistance Profile in Relation to Phylogenetic Background among Commensal *Escherichia coli* Derived from Various Mammals

KATARZYNA BALDY-CHUDZIK* and MICHAŁ STOSIK

Department of Microbiology and Genetics, Institute of Biotechnology and Environmental Science, University of Zielona Góra, Zielona Góra, Poland

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Abstract

The paper describes the prevalence of resistant strains within the genetic structure of *E. coli* (phylogenetic group A, B1, B2 and D). A total of 200 commensal *E. coli* strains have been derived from 10 species of healthy animals residing on ZOO Safari Park area, in Świerkocin, Poland. The phylogenetic structure of *E. coli* has been analysed with the use of a PCR-based method. The strains were tested in terms of their susceptibility to eight classes of antibiotics: aminoglycosides, penicillins, cephalosporins, tetracyclines, nitrofurans, sulphonamides, phinicols, and quinolones. The genetic structure of *E. coli* revealed a not uniform distribution of strains among the four phylogenetic groups with significantly numerous representation of groups A and B1. Resistant *E. coli* were found within each of the phylogenetic groups. Strains resistant to one class of antibiotics occurred significantly more frequently in phylogenetic groups B2 and D (potential pathogens), whereas strains resistant to more than one class of antibiotics belonged to phylogenetic groups A and B1 (typical commensals) in a prevailing number of cases.

Key words: commensal *E. coli*, phylogenetic groups of *E. coli*, resistance to antibiotics

Introduction

Commensal bacteria inhabiting human and animal intestine, *E. coli* among others, are subjected to contact with various antibiotics applied at various concentrations and with varied frequency. Antimicrobial agent resistance genes are situated in mobile genetic elements such as plasmids, transposons and integrons (Carattoli, 2001; Rowe-Magnus and Mazel, 1999). Once acquired resistance genes can be transferred between bacteria. The host organism's selective pressure selects the resistant bacteria that have specific patterns of resistance (Sayah *et al*., 2005). The observations of the development of resistant bacteria have resulted in hypothesis that commensal bacteria serve as a reservoir of resistance genes (Wray and Gnanou, 2000). The research on the prevalence of the resistance between natural *E. coli* in various mammals may provide arguments supporting the hypothesis. The arguments confirming such a possibility are as follows: 1) common occurrence of *E. coli* as an element of intestine microflora in mammals; 2) the occurrence, within *E. coli* species, of pathogens causing both intestinal and extraintestinal diseases in humans and animals; 3) the genetic structure of *E. coli*, which is of a clonal character and is composed of 4 main phylogenetic groups A, B1, B2 and D. This is evidence that pathogenic *E. coli* originate from commensal strains revealing diversified preference to the acquisition of certain virulence factors. *E. coli* of phylogenetic group B2 accumulate extraintestinal virulence factors. Enteropathogenic *E. coli* are assigned to group D in prevailing number of cases. Groups A and B1 are determined as typical commensals (Duriez *et al*., 2001; Reid *et al.*, 2000). Thus, the question of the prevalence of the resistance within the four main phylogenetic groups is raised in the aspect of the diversified genetic structure of *E. coli*. It seems that mammals, which are kept in zoological gardens and safari parks may serve as good model objects for

* Corresponding author: K. Baldy-Chudzik, Department of Microbiology and Genetics, Institute of Biotechnology and Environmental Science, University of Zielona Góra, Monte Cassino 21B, 65-561 Zielona Góra, Poland; phone (48) 68 3287333; fax: (48) 68 3287323; **e-mail: K. Baldy-Chudzik@ibos.uz.zgora.pl**

such research, as they stay in a limited area for a long time and are under continuous control.

Our research involved the analysis of commensal *E. coli* derived from carnivorous and herbivorous mammals staying in the grounds of ZOO Safari Park in Świerkocin. The aim of the research was to analyse the prevalence of resistant strains within the four main phylogenetic groups of *E. coli*.

Experimental

Materials and Methods

The source of strains. The material was derived from adult, healthy individuals. The source organisms were five species of herbivorous animals from the taxonomic order *Artiodactyla*: waterbuck (*Kobus ellipsiprymus*), eland (*Taurotragus oryx*), yak (*Bos mutus graniens*), aurochs (*Bos primigenius*), buffalo (*Bubalus bubalis)* and five species from the taxonomic order *Carnivora:* lion (*Panthera leo*), lynx (*Felis lynx*), wildcat (*Felis silvetris*), racoon (*Procyon lotor*), dingo (*Canis familiaris dingo*). The research included both herbivorous and carnivorous species because of the different diets and the different layout of the walks in the Zoo's area. The buffaloes and the yaks walks were separated with a walk common for aurochs, eland and waterbuck. The walks of the carnivorous animals were isolated from both other carnivorous as well as herbivorous animals.

Identification of *E. coli*. The samples were drawn once. Each of the 10 tested animal species was represented by samples drawn from three individuals. Thus, a total of 30 faecal samples were obtained. *E. coli* were isolated from each individual faecal sample. Inoculation was performed onto m-FC agar with rosolic acid (Merck). After 24 h of incubation at 44.5°C blue colonies (randomly 15 colonies from each sample) were passaged onto MacConkey agar plates (Difco). Lacto-positive isolates were verified with a series of tests IMV and C (indole, methyl red, Voges-Proskauer, citrate) just as described earlier (Baldy-Chudzik and Stosik, 2003). All *E. coli* isolates were stored at –70°C in Luria-Bertani (LB) broth containing 25% glycerol. For the experiments, the strains were cultured in LB broth for 18 h at 37°C.

DNA template preparation. A single bacterial colony was suspended in 25 µl sterile water then heated to 99°C, 10 min, then cooled and centrifuged. The obtained supernatant was the source of DNA template for PCR reaction.

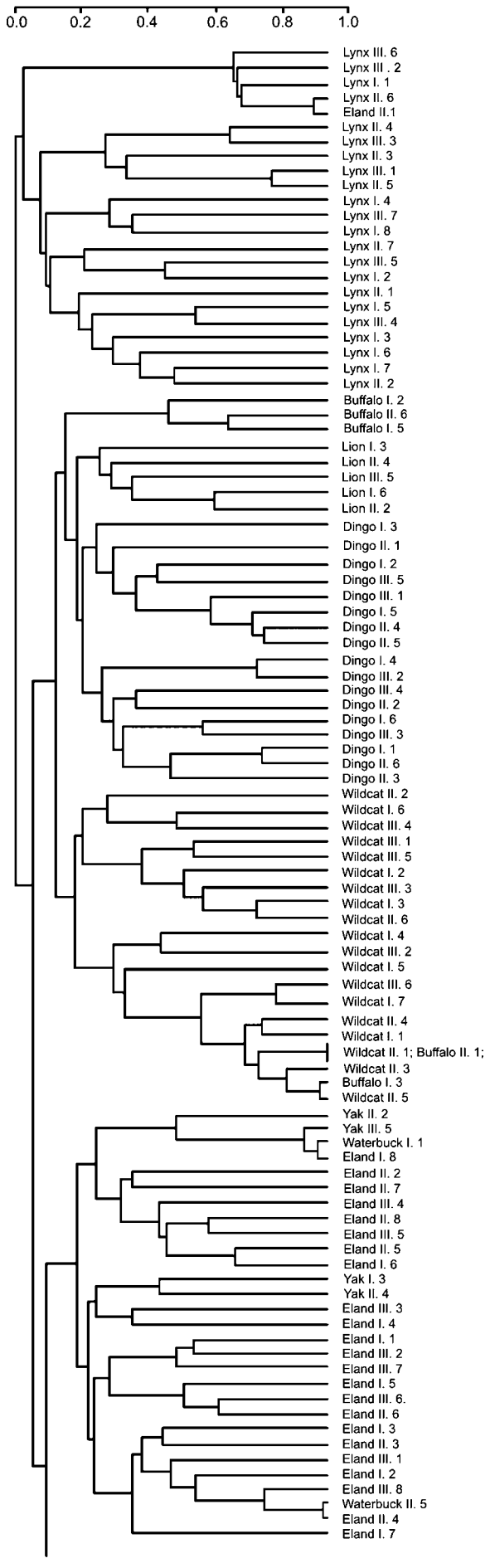
BOX-PCR DNA fingerprinting. All the isolates identified in IMV and C tests as *E. coli* were used to DNA rep-PCR fingerprinting with BOX A1R primer (BOX-PCR). The primer sequence BOX A1R and the amplification conditions were used according to the ones described earlier (Baldy-Chudzik and Stosik, 2005). BOX-PCR products were analysed electrophoretically in 0.8% (w/v) agarose in 1xTBE buffer and stained with ethidium bromide. Gels were documented as TIFF files and analysed with Fingerprinting II informatix software (BioRad). BOX-PCR gel lanes were normalized using 1 kb DNA Ladder (Fermentas), as external reference standards. The similarity matrices were calculated based on Pearson's similarity coefficient with 1.5% tolerance for the position of a band. Cluster analysis of similarity matrix was performed by the unweighted pair group method using arithmetic averages (UPGMA). The correlations were expressed as the percentage of similarity. The comparative analysis of BOX-PCR patterns generated by repeated analyses of strain *E. coli* K12 (CIP, Paris, France) (n = 50), revealed similarity >90%. On this basis, the similarity of BOX-PCR patterns of order 85% was established as a cut-off value for determination of unique strains. The isolates, which were derived from hosts of the same species and which revealed the similarity of BOX-PCR pattern >85% were regarded to be the same and were eliminated from subsequent analyses. Based on the similarity analysis of genomic patterns, 200 unique *E. coli* strains were selected for the subsequent studies (Fig. 1, Table I).

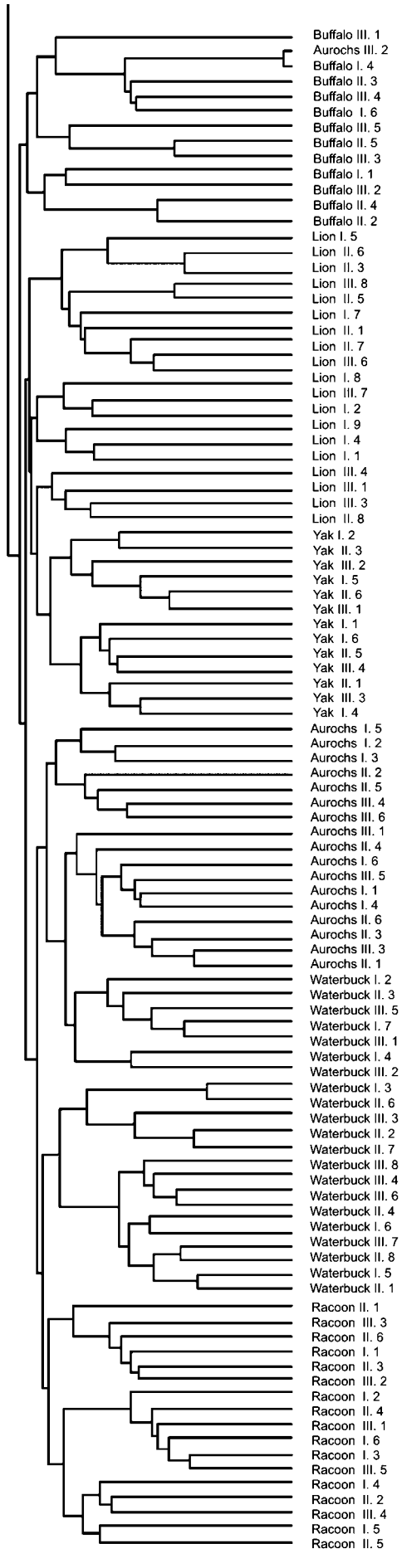
Determination of phylogenetic groups. The method used three pairs of primers of PCR reaction of sequences homologic to genetic markers specific for phylogenetic groups of *E. coli*: gene *chuA*, gene *yjaA*, and an anonymous DNA sequence TspE4C2. The PCR primers, the amplification steps and the electrophoretic analysis were all used according to those given by Clermont *et al*. (2000). On the basis of a specific profile of PCR products, the determination of the phylogenetic group was carried out in the following way: *chuA*$^+$, *yja*A$^+$, group B2; *chuA*$^+$, *yja*A$^-$, group D; *chuA*$^-$, TspE4.C2$^+$, group B1; *chuA*$^-$, TspE4.C2$^-$, group A.

Antimicrobial agents susceptibility. Antibiograms and their interpretation were made using the disk diffusion method following the CLSI (formerly NCCLS) standards (National Committee for Clinical Laboratory

Fig. 1. Dendrogram of the similarity relation of BOX-PCR fingerprinting patterns of the 200 unique *E. coli* strains derived from ten source species.

Each of the isolates is defined by: taxonomic species of the host / I, II or III refers to an individual of a given species, an Arabic numeral refers to a number of an isolate identified in an individual.


0.0
0.2
0.4
0.6
0.8
1.0
Lynx III. 6
Lynx III . 2
Lynx I. 1
Lynx II. 6
Eland II.1
Lynx II. 4
Lynx III. 3
Lynx II. 3
Lynx III. 1
Lynx II. 5
Lynx I. 4
Lynx III. 7
Lynx I. 8
Lynx II. 7
Lynx III. 5
Lynx I. 2
Lynx II. 1
Lynx I. 5
Lynx III. 4
Lynx I. 3
Lynx I. 6
Lynx I. 7
Lynx II. 2
Buffalo I. 2
Buffalo II. 6
Buffalo I. 5
Lion I. 3
Lion II. 4
Lion III. 5
Lion I. 6
Lion II. 2
Dingo I. 3
Dingo II. 1
Dingo I. 2
Dingo III. 5
Dingo III. 1
Dingo I. 5
Dingo II. 4
Dingo II. 5
Dingo I. 4
Dingo III. 2
Dingo III. 4
Dingo II. 2
Dingo I. 6
Dingo III. 3
Dingo I. 1
Dingo II. 6
Dingo II. 3
Wildcat II. 2
Wildcat I. 6
Wildcat III. 4
Wildcat III. 1
Wildcat III. 5
Wildcat I. 2
Wildcat III. 3
Wildcat I. 3
Wildcat II. 6
Wildcat I. 4
Wildcat III. 2
Wildcat I. 5
Wildcat III. 6
Wildcat I. 7
Wildcat II. 4
Wildcat I. 1
Wildcat II. 1; Buffalo II. 1;
Wildcat II. 3
Buffalo I. 3
Wildcat II. 5
Yak II. 2
Yak III. 5
Waterbuck I. 1
Eland I. 8
Eland II. 2
Eland II. 7
Eland III. 4
Eland II. 8
Eland III. 5
Eland II. 5
Eland I. 6
Yak I. 3
Yak II. 4
Eland III. 3
Eland I. 4
Eland I. 1
Eland III. 2
Eland III. 7
Eland I. 5
Eland III. 6.
Eland II. 6
Eland I. 3
Eland II. 3
Eland III. 1
Eland I. 2
Eland III. 8
Waterbuck II. 5
Eland II. 4
Eland I. 7


Buffalo III. 1
Aurochs III. 2
Buffalo I. 4
Buffalo II. 3
Buffalo III. 4
Buffalo I. 6
Buffalo III. 5
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Buffalo III. 3
Buffalo I. 1
Buffalo III. 2
Buffalo II. 4
Buffalo II. 2
Lion I. 5
Lion II. 6
Lion II. 3
Lion III. 8
Lion II. 5
Lion I. 7
Lion II. 1
Lion II. 7
Lion III. 6
Lion I. 8
Lion III. 7
Lion I. 2
Lion I. 9
Lion I. 4
Lion I. 1
Lion III. 4
Lion III. 1
Lion III. 3
Lion II. 8
Yak I. 2
Yak II. 3
Yak III. 2
Yak I. 5
Yak II. 6
Yak III. 1
Yak I. 1
Yak I. 6
Yak II. 5
Yak III. 4
Yak II. 1
Yak III. 3
Yak I. 4
Aurochs I. 5
Aurochs I. 2
Aurochs I. 3
Aurochs II. 2
Aurochs II. 5
Aurochs III. 4
Aurochs III. 6
Aurochs III. 1
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Aurochs I. 6
Aurochs III. 5
Aurochs I. 1
Aurochs I. 4
Aurochs II. 6
Aurochs II. 3
Aurochs III. 3
Aurochs II. 1
Waterbuck I. 2
Waterbuck II. 3
Waterbuck III. 5
Waterbuck I. 7
Waterbuck III. 1
Waterbuck I. 4
Waterbuck III. 2
Waterbuck I. 3
Waterbuck II. 6
Waterbuck III. 3
Waterbuck II. 2
Waterbuck II. 7
Waterbuck III. 8
Waterbuck III. 4
Waterbuck III. 6
Waterbuck II. 4
Waterbuck I. 6
Waterbuck III. 7
Waterbuck II. 8
Waterbuck I. 5
Waterbuck II. 1
Racoon II. 1
Racoon III. 3
Racoon II. 6
Racoon I. 1
Racoon II. 3
Racoon III. 2
Racoon I. 2
Racoon II. 4
Racoon III. 1
Racoon I. 6
Racoon I. 3
Racoon III. 5
Racoon I. 4
Racoon II. 2
Racoon III. 4
Racoon I. 5
Racoon II. 5

Table I
Genetic structure of commensal *E. coli* strains derived from different animal species

Source species		Number of *E. coli* from individuals:			All number n	Number/(%) of *E. coli* in phylogenetic groups:			
		I	II	III		A	B1	B2	D
Carnivorous:	lion	9	8	8	25	10/40.0	8/32.0	3/12.0	4/16.0
	lynx	8	7	7	22	10/45.4	6/27.3	3/13.6	3/13.6
	racoon	6	6	5	17	6/35.2	5/29.4	3/17.6	3/17.6
	dingo	6	6	5	17	8/47.1	5/29.4	0/0	4/23.5
	wildcat	7	6	6	19	6/31.6	6/31.6	4/21.0	3/15.8
Herbivorous:	buffalo	6	6	5	17	5/29.4	7/41.8	3/17.6	2/11.8
	waterbuck	8	8	8	24	7/29.2	9/37.5	5/20.8	3/12.5
	aurochs	6	6	6	18	5/27.8	8/44.4	3/16.7	2/11.1
	eland	8	8	8	24	8/33.3	10/41.7	3/12.5	3/12.5
	yak	6	6	5	17	4/23.5	8/47.1	3/17.6	2/11.8
	all				200	69/34.5	72/36.0	30/15.0	29/14.5

Standards, 2003) for 14 antimicrobial agents (Table II). These antimicrobial agents were selected on the basis of their importance in treating human or animal bacterial infections and their use as feed additives to feed efficiency and/or disease prophylaxis in animals and on the basis of their ability to provide diversity for representation of different antimicrobial agent classes. Each of *E. coli* isolate was grown overnight in LB broth at 37°C and diluted with LB broth to an absorbance at 600 nm of ~0.1. The diluted *E. coli* inoculum was swabbed onto a Mueller-Hinton agar (Difco) plate. Fourteen commercially prepared antimicrobial agent disks (Becton Dickinson) were placed on the inoculated plates. The plates were incubated at 35°C for 18 to 20 h. The diameters (in millimeters) of the clear zones of growth inhibition around the antimicrobial agent disks, including the 6-mm disk diameter, were measured by using precision callipers. The breakpoints used to categorize isolates as resistant or not resistant to each antimicrobial agent were those recommended by manufacturer (BBL Sensi-Disc Antimicrobial Susceptibility Test Discs, Becton Dickinson) for *E. coli* (Table II). Intermediate zones of inhibition were counted as sensitive for purposes of this study. *E. coli* ATCC 25922 was used for quality control strain.

Table II
Concentrations and diffusion zone breakpoints for resistance for antimicrobial agents in this study, sorted by class of antimicrobial agent

Antimicrobial agent	Drug code	Disk drug concentrations (μg)	Diffusion zone breakpoint (mm)
AMINOGLYCOSIDES:			
neomycin	N	30	≤12
gentamicin	GM	10	≤12
streptomycin	S	10	≤11
amikacin	AN	30	≤14
PENICILLINS:			
ampicillin	AM	25	≤13
amoxicillin/clavulanic acid	AMC	20/10	≤13
CEPHALOSPORINS:			
cephalothin	CF	30	≤14
cefoperazone	CFP	75	≤15
TETRACYCLINES:			
tetracycline	TE	30	≤14
doxycycline	D	30	≤12
NITROFURANS:			
nitrofurantoin	FT	300	≤14
SULPHONAMIDES:			
trimethoprim/sulfamethoxazole	SXT	1.25/23.75	≤10
PHINICOLS:			
chloramphenicol	C	30	≤12
QUINOLONES:			
nalidixic acid	NA	30	≤13

Statistical analysis. Chi-square was used for the comparisons between groups (Sneath and Sokal, 1973).

Results

A total number of 200 *E. coli* strains were identified, among which 100 were derived from carnivorous and 100 from herbivorous animals. The analysis of the genetic structure of *E. coli* isolates showed significant differences between strains derived from carnivorous and herbivorous animals (Table I). Strains derived from carnivorous animals occurred in groups A and D significantly more frequently than the ones derived from herbivorous animals ($p<0.001$, $p<0.01$ respectively). *E. coli* from herbivorous species were significantly more frequently classified to group B1 ($p<0.001$). The observed diversity of the genetic structures of *E. coli* between carnivorous and her-

Table III
Occurrence of resistant *E. coli* in the examined animal species

The class of agent (antibiotic)		% of resistant *E. coli* for each animal species										
		Total n = 200	Carnivorous:					Herbivorous:				
			Lion n = 25	Lynx n = 22	Raccoon n = 17	Dingo n = 17	Wildcat n = 19	Buffalo n = 17	Waterbuck n = 24	Aurochs n = 18	Eland n = 24	Yak n = 17
Aminoglycosides:	neomycin	1.5	4	0	0	5.9	5.3	0	0	0	0	0
	gentamicin	3	4	9.1	0	0	5.3	5.9	0	0	4.2	0
	streptomycin	22.5	36	36.4	29.4	29.4	42.1	17.6	8.4	0	20.8	0
	amikacin	3.5	8	9.1	0	11.8	0	0	0	0	4.2	0
Penicillins:	ampicillin	13.5	20	9.1	11.8	29.4	42.1	5.9	4.2	0	12.5	0
	amoxicillin/ clavulanic acid	3	4	4.6	0	0	5.3	5.9	0	0	8.4	0
Cephalosporins:	cephalothin	40	56	36.4	47.1	29.4	31.6	52.9	41.7	66.7	29.2	5.9
	cefoperazone	22	24	22.7	41.2	29.4	26.3	11.8	20.8	27.8	12.5	5.9
Tetracyclines:	tetracycline	38.5	44	50	47.2	11.8	42.1	52.9	41.7	16.8	50	17.6
	doxycycline	15	20	13.6	17.6	11.8	21.1	11.8	29.2	5.6	12.5	0
Nitrofurans:	nitrofurantoin	9	8	9.1	17.6	11.8	15.8	17.6	8.4	0	4.2	0
Sulphonamides:	rimethoprim/ sulfamethoxazole	49	20	50	58.8	47.1	47.4	52.9	50	83.3	62.5	23.5
Phenicols:	chloramphenicol	3.5	20	0	0	5.9	5.3	0	0	0	0	0
Quinolones:	nalidixic acid	9.5	20	0	17.6	23.5	26.3	11.8	0	0	0	0

bivorous species may be explained with different diet requirements (resulting from their taxonomic position). Diet has been reported to be the key factor determining the relative abundance of *E. coli* phylogenetic groups in mammals (Gordon and Crowling, 2003). The revealed higher homogeneity in the genetic structure of *E. coli* in herbivorous species has resulted from the fact that the examined species were all ruminants rather than from the incidences of transmission of *E. coli* between them. The conclusion is supported by the results of BOX-PCR fingerprints, where the genomic similarity (>80%) proving the transmission of strains, has been revealed in individual *E. coli* derived from: lynx and eland; wildcat and buffalo; yak, waterbuck, and eland as well as buffalo and aurochs (Fig. 1).

Among the 200 examined strains, 74 were susceptible to all the antimicrobial agents tested. Resistances to cephalosporins, tetracyclines, and sulphonamides were generally most frequent (Table III). Between *E. coli* from herbivorous animals, 65% of strains from yak were susceptible to all the antibiotics tested, whereas 38, 35, 29, and 17% of *E. coli* from eland, buffalo, waterbuck, and aurochs showed no resistance at all, respectively. In carnivorous animals, 47% of *E. coli* from dingo, 37% from wildcat, 36% from lion and lynx, and 35% from raccoon were susceptible to all the antibiotics tested. Strains from carnivorous animals were more frequently resistant to aminoglycosides ($p<0.001$), penicillins ($p<0.001$), as well as nitrofurans ($p<0.001$) and quinolones ($p<0.001$) in comparison to *E. coli* from herbivorous animals. *E. coli* multi-resistant to antibiotics belonging both to the same class as well as various classes were found in carnivorous animals more frequently than in herbivorous ones. One strain from wildcat presented resistance to 12 antimicrobial agents (N, GM, S, AM, AMC, CF, CFP, TE, FT, SXT, C, and NA). Among strains of all the herbivorous species, the most frequently identified multi-resistance pattern comprised the following antibiotics: CF, TE, and SXT. The same resistance pattern was identified also in three strains derived from lynx, what proves the transmission of resistance factors between animals living in a neighborhood. Among multi-resistant *E. coli*, simultaneous resistance to cephalosporins (CF or/and CFP) and AM, characteristic for penicillins, was found in 26 strains among which 17 were derived from carnivorous. Simultaneous resistance to cephalosporin and AM was shown in five *E. coli* whose multi-resistance patterns comprised five classes of the applied antibiotics. Resistance to TE, D or SXT exclusively, occurred with a comparable frequency in *E. coli* from herbivorous and carnivorous animals. The resistance pattern comprising SXT and D or SXT and TE occurred in both herbivorous and carnivorous, whereas resistance to SXT and CF was a characteristic feature for herbivorous animals exclusively.

Strains from carnivorous animals differed from *E. coli* of herbivorous both in the genetic structure and the resistance patterns. The observed differences constituted the base for the following generalizations:

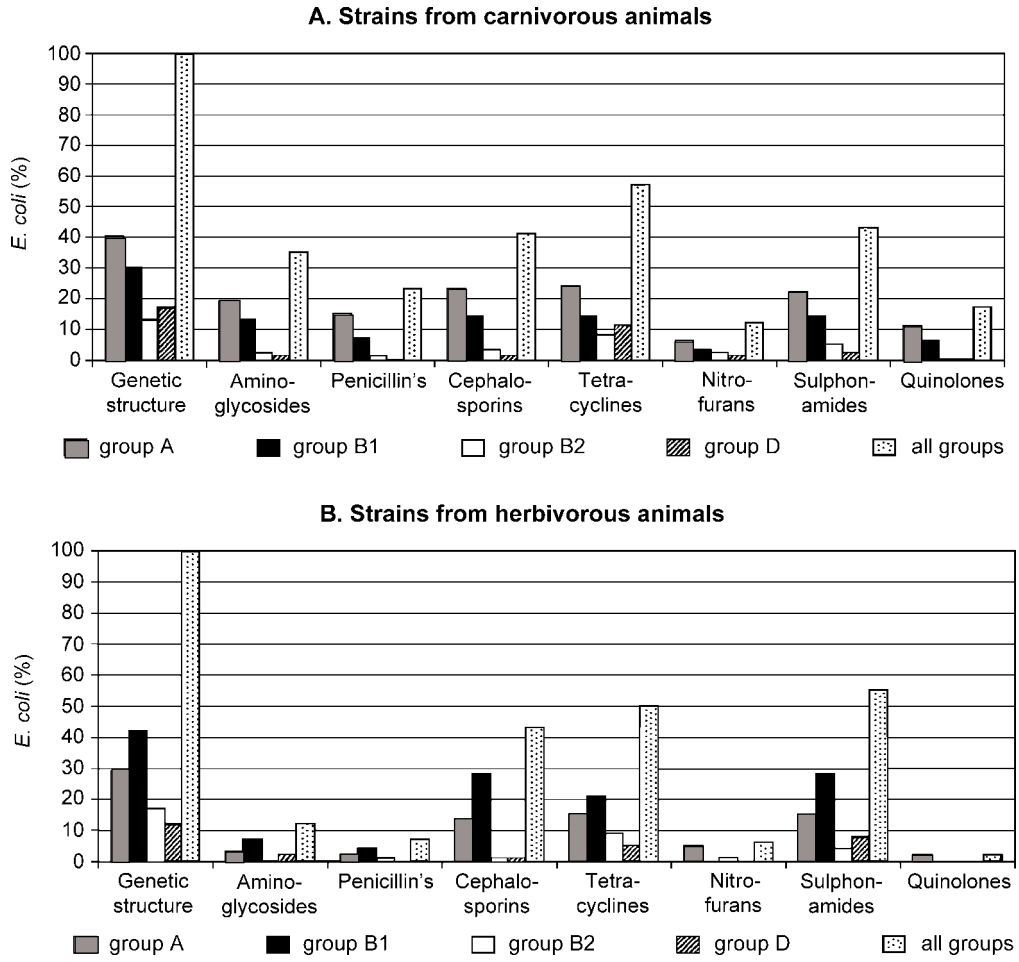

Fig. 2. Genetic structure of *E. coli* resistant to the examined classes of antibiotics in comparison to the genetic structure of *E. coli* obtained from carnivorous (A) and herbivorous (B) animals respectively.

1) strains derived from carnivorous and herbivorous species were treated as two separate sets, without specifying the source species. 2) the resistance was later analyzed with regard to a class not to a particular antibiotic since strains multi-resistant to antibiotics of the same class occurred more frequently. What is more, because of a low percentage of *E. coli* resistant to chloramphenicol (phinicols), the class was neglected in subsequent analyses. The analysis of the genetic structure of resistant *E. coli* showed that a prevailing percentage of strains resistant to any class of the applied antibiotics belonged to phylogenetic groups A and B1 (typical commensals), derived from both carnivorous and herbivorous animals (Fig. 2). Not numerous resistant *E. coli* from groups B2 and/or D usually co-occurred with the resistant strains from groups A and B1.

The susceptible strains constituted 36% of the set from herbivorous animals and 38% of set of *E. coli* derived from carnivorous animals (Fig. 3). A high percentage of multi-resistant strains were revealed in each of the sets. Among *E. coli* from herbivorous animals 24% strains revealed resistance to three classes of antibiotics, whereas 38% strains revealed resistance to more than three classes of antibiotics in the set from carnivorous animals (from 4 up to 7 classes) (Fig. 3). The genetic structure of multi-resistant strains revealed distinct features common for both analyzed sets of *E. coli*: 1) strains resistant to one class of antibiotics were represented by groups B2 and D (potential pathogens) whereas the representation of strains from group A and B1 did not occur (typical commensals); 2) strains resistant to two and three classes of antibiotics were represented by all the four phylogenetic groups; 3) strains resistant to more than three classes of antibiotics in vast majority belonged to group A and B1 and the representation of group B2 was insignificant or did not occur, and group D did not occur.

Discussion

The highest levels of resistance were observed for sulfonamides, tetracyclines, and cephalosporins in strains derived from all animal species. Resistance to

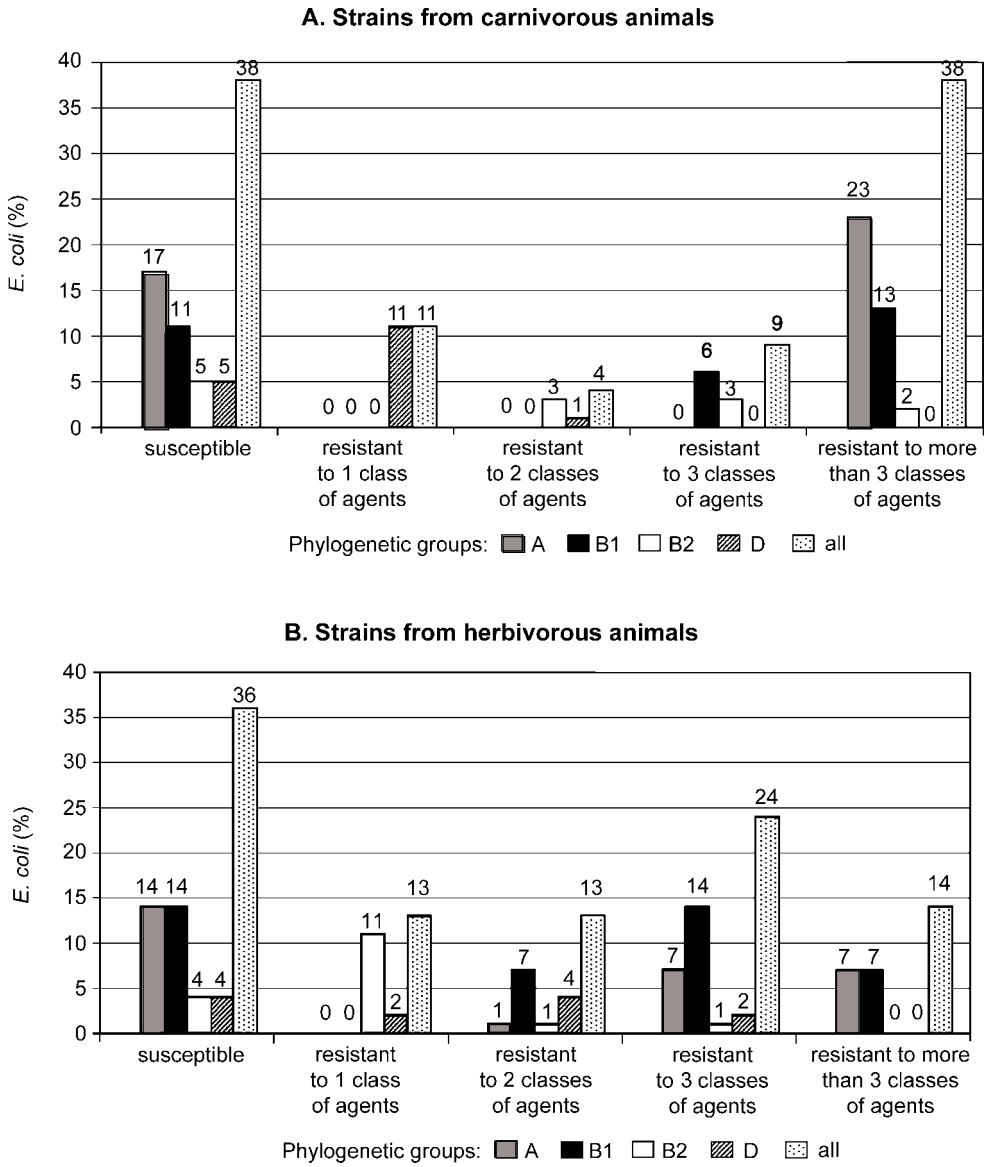

Fig. 3. Genetic structure of susceptible and multi-resistant *E. coli* derived from carnivorous (A) and herbivorous (B) animals. Values above the bar correspond to the number of *E. coli* strains in each of phylogenetic group.

sulphonamides and tetracyclines was shown in each of the phylogenetic groups of *E. coli*, but strains resistant to a single antibacterial agent (sulphonamide or tetracycline) belonged exclusively to phylogenetic groups B2 and D (potential pathogens). The prevalence of resistance to these classes of antibiotics could be the result of widespread and lengthy use of these antimicrobial agents in non-food producing animals (Klein and Bulte, 2003). Resistance to tetracycline is plasmid mediated. The large numbers of genetic determinants for tetracycline resistance make it more possible for a susceptible bacterium to acquire resistance factors than if only a few determinants were available (McEwen and Fedorka-Cray, 2002). Resistance to sulfonamides is plasmid mediated, but chromosomal mutations for sulfonamide resistance take place very seldom. Resistance to sulfonamides is widespread in the environment and cross-resistance between sulfonamides is complete (Sköld, 2001).

A high level of resistance to cephalosporins was observed in both carnivorous and herbivorous animals, whereas resistance to penicillins occurred considerably more frequently in carnivorous animals. The resistance to these classes of antibiotics as well as to the remaining examined classes co-occurred, forming patterns of multi-resistance in strains from phylogenetic groups A and B1. Cefoperazone (the third generation of cephalosporins) is used in veterinary medicine for various species of animals. The first-generation cephalosporin's – cephalothin are heavily used for the treatment of bacterial infections and particularly of urinary tract infections in cats and dogs but rarely or never in ruminants (all herbivorous animals in this study are ruminant) (Lanz *et al.*, 2003;

Donaldson *et al*., 2006). In herbivorous animals, the resistance against the third-generation of cephalosporins can develop resistance to cephalothin. The most common mechanism of resistance to β-lactam antibiotics (including the tested penicillins, and cephalosporins) is the production of various β-lactamases, which hydrolyze β-lactam ring. The *E. coli* resistance may be caused by both mutations in *ampC* gene (conditioning constitutive β-lactamase) located on chromosomes, and a broad spectrum of β-lactamase genes located on plasmids. AmpC β-lactamases are not inhibited by inhibitors such as clavulanic acid. The plasmid resistance is the effect of a stable mutation and is easy to maintain by bacteria even at the absence of the selective pressure of antibiotics. β-lactamases located on a plasmid are sensitive to inhibitors such as clavulanic acid (Livermore, 1995; Siu *et al*., 2003). The family of β-lactamases is numerous, and growing. Among strains of a complex resistance patterns including: penicillins, penicillins with clavulanic acid and cephalosporins, a univocal identification of types of developed resistance may be achieved by specific gene identification. It is the consequence of the fact that a simultaneous occurrence of both chromosomal and plasmid genes conditioning β-lactometers are found more and more frequently in multi-resistant strains (Tenover *et al*., 2003). The differences in resistance patterns between carnivorous and herbivorous animals may be caused by exposure to different agents because of differences in the husbandry of these species or other factors that may have increased or decreased the likelihood of the development and conservation of resistant bacteria in the animal species. For example, resistance to quinolones occurred considerably more frequently in carnivorous than in herbivorous. The resistance to this class of antibiotics is the result of chromosomal mutations, and not the result of acquiring genes from other bacteria of the same or other species, and the occurrence of resistance is conditioned by the frequency of applying the medicine (Chen *et al*., 2001).

The research comprised 10 species of healthy mammals with the aim to analyze the genetic structure of antibiotic-resistant commensal *E. coli*. Such direct comparative analyses of *E. coli* in various host species are rare in the literature and are usually concerned with clinical strains derived from a single host species (Hill *et al.*, 2003; Selander *et al*., 1986). The obtained results are essential for the better recognition of population biology of *E. coli*, because they indicate the fact that within a genetic structure of *E. coli* of various source species, phylogenetic groups A and B1, *i.e.* typical commensal strains, compose a basic reservoir of multi-resistant strains. They also suggest that *E. coli* from groups A and B1 occupy similar niches in the organisms of the examined animals. In such niches, gradual acquiring of resistance factors may result in increased surviving. It has been reported that multi-resistant *E. coli* survive better beyond the host organism than the susceptible strains (Abu-Ghazaleh, 2001). The contamination of the habitat of animals with multi-resistant *E. coli* increases the real hazard of the prevalence of both such strains as well as the resistance factors. The prevalence of multi-resistant *E. coli* from groups A and B1 in the environment may be essential for the recently reported failures in the treatment of extraintestinal infections of *E. coli* in humans (particularly reoccurring infections of urinary tract in females) (Johnson *et al.*, 2005; Moreno *et al*., 2006). The problems in the treatment resulted from the occurrence of opportunistic, multi-resistant *E. coli* from groups A and B1 rather than uropathogenic *E. coli* from group B2, while in most cases uropathogenic *E. coli* from group B2 constituted a bacterial subpopulation sensitive to antibiotics.

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