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The Phenotypic and Genotypic Characteristics of Antibiotic Resistance in *Escherichia coli* Populations Isolated from Farm Animals with Different Exposure to Antimicrobial Agents

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Abstract

The aim of the study was to determine the influence of the presence or the absence of antibiotic input on the emergence and maintenance of resistance in commensal bacteria from food producing animals. The research material constituted *E. coli* isolates from two animal species: swine at different age from one conventional pig farm with antibiotic input in young pigs and from beef and dairy cattle originated from organic breeding farm. The sensitivity to 16 antimicrobial agents was tested, and the presence of 15 resistance genes was examined. In *E. coli* from swine, the most prevalent resistance was resistance to streptomycin (88.3%), co-trimoxazole (78.8%), tetracycline (57.3%) ampicillin (49.3%) and doxycycline (44.9%) with multiple resistance in the majority. The most commonly observed resistance genes were: bla_{TEM} (45.2%), *tetA* (35.8%), *aadA1* (35.0%), *sul3* (29.5%), *dfrA1* (20.4%). Differences in phenotypes and genotypes of *E. coli* between young swine undergoing prevention program and the older ones without the antibiotic pressure occurred. A disparate resistance was found in *E. coli* from cattle: cephalothin (36.9%), cefuroxime (18.9%), doxycycline (8.2%), nitrofurantoin (7.7%), and concerned mainly dairy cows. Among isolates from cattle, multidrug resistance was outnumbered by resistance to one or two antibiotics and the only found gene markers were: bla_{SHV} (3.4%), *tetA* (1.29%), bla_{TEM} (0.43%) and *tetC* (0.43%). The presented outcomes provide evidence that antimicrobial pressure contributes to resistance development, and enteric microflora constitutes an essential reservoir of resistance genes.

Key words: *Escherichia coli*, antibiotic resistance, resistance genes, animal production

Introduction

The increase of antibiotic resistance raises general concerns. Research focuses mainly on clinically important cases, nonetheless this issue involves all environmental areas. *Escherichia coli* is a commensal bacterium with primary habitat in the intestinal tract of humans and animals like swine, cattle and poultry which are used for food production (Hammerum and Heuer, 2009). *E. coli* can possess some virulence factors that allow a variety of intestinal and extraintestinal infections to appear, such as diarrhea, urinary tract infection, meningitis, septicemia or pneumonia both in humans and animals (Donnenberg *et al*., 2002; Hammerum and Heuer, 2009; Smith *et al*., 2007; Touchon *et al*., 2009). The significance of *E. coli* as a health hazard also arises from its dissemination capabilities. Strains from food-producing animals can contaminate meat products during slaughter, can survive a few days in a chiller and enter the food chain (Delsol *et al*., 2010; Tw *et al*., 2010). Various studies have demonstrated that resistant strains of animal origin are able to colonize or cause human infections (Aarestrup *et al*., 2008; Bélanger *et al*., 2011; Collignon *et al*., 2009; Manges and Johnson, 2012). The source of this problem can be food animal production systems. Intense application of antibiotics in conventional breeding of food animals, and also the volume of meat production results in the fact that the commensal flora in animals intestinal track constitutes an essential reservoir of resistance genes. Transfer of resistance determinants by mobile genetic elements is an important factor that can contribute to an increase in multiresistant bacteria (Blake *et al*., 2003). Organic breeding of food animals differs from conventional in many ways. In general, organic breeding standards prohibit the use of chemical fertilizers, chemicals and pharmaceuticals. Organic farming is based on the support of 'natural processes' in their production systems. The aim of this study was to compare the impact of conventional and organic animal production systems on the emergence and the development of resistance in *E. coli* strains.

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Experimental

Materials and Methods

Source of isolates. *E. coli* isolates from fecal samples derived from cattle and swine constituted the research material. Cattle (84 individuals) came from organic breeding farm and were classified into three distinct populations: two herds of beef cows feeding on pastures (labeled: I, II) and dairy cows housed in a barn. Swine derived from one conventional farm were represented by four age groups (25 individuals in each): group I comprised 6-week-old piglets (called piglets I), group II included 8-week-old piglets (piglets II), group III comprised 5-month-old sows (sows I), and group IV comprised 7-month-old sows (sows II). Conventional swine farming includes a post-weaning medical program involving treatment with sulfonamide, co-trimoxazole (trimethoprim/sulfamethoxazole) and ampicillin. The program was applied to all individuals in their 6th week.

Isolation and identification. *E. coli* was isolated from feces and identification was carried out with biochemical testing, also BOX-PCR fingerprinting was conducted (Baldy-Chudzik and Stosik, 2007). The final material for the study consisted of 274 non-identical *E. coli* isolates from swine and 233 from cattle.

Antimicrobial susceptibility testing. Antibiotic sensitivity was determined using disc diffusion method on Mueller Hinton agar (Merck), following CLSI standards (CLSI, 2010). *E. coli* ATCC 25922 was used as a reference strain. The following antibiotics were tested: ampicillin (10 µg), amoxicillin/ clavulanic acid

Table I
PCR conditions for detection antimicrobial resistance genes in *E. coli* isolates.

Target gene	Primer name	Primer sequence (5'-3')	Primer concentration	Annealing temperature (°C)	Product size (bp)	Reference
bla_{TEM}	GKTEM-F GKTEM-R	TTAACTGGCGAACTACTTAC GTCTATTTCGTTCATCCATA	0,2 µM	55	247	Kozak *et al.*, 2009
bla_{SHV}	SHV-F SHV-R	AGGATTGACTGCCTTTTTG ATTTGCTGATTTCGCTCG	0,4 µM	55	393	Kozak *et al.*, 2009
bla_{CMY-2}	CMY- F CMY-R	GACAGCCTCTTTCTCCACA TGGACACGAAGGCTACGTA	0,2 µM	55	1000	Kozak *et al.*, 2009
tet A	Tet A-F Tet A-R	GCTACATCCTGCTTGCCTTC CATAGATCGCCGTGAAGAGG	0,2 µM	60	210	Ng *et al.*, 2001
tet B	Tet B-F Tet B-R	TTGGTTAGGGGCAAGTTTTG GTAATGGGCCAATAACACCG	0,2 µM	60	659	Ng *et al.*, 2001
tet C	Tet C-F TetC-R	CTTGAGAGCCTTCAACCCAG ATGGTCGTCATCTACCTGCC	0,2 µM	60	418	Ng *et al.*, 2001
tet D	Tet D-F Tet D- R	AAACCATTACGGCATTCTGC GACCGGATACACCATCCATC	0,2 µM	60	787	Ng *et al.*, 2001
tet M	Tet M- F Tet M- R	GTGGACAAAGGTACAACGAG CGGTAAAGTTCGTCACACAC	0,2 µM	58	406	Ng *et al.*, 2001
aadA1	aadA- F aadA-R	GTGGATGGCGGCCTGAAGCC AATGCCCAGTCGGCAGCG	0,1 µM	63	525	Kozak *et al.*, 2009
strA/*strB*	strA-F strB-R	ATGGTGGACCCTAAAACTCT CGTCTAGGATCGAGACAAAG	0,4 µM	63	893	Kozak *et al.*, 2009
aac(3)IV	aac4-F aac4-R	TGCTGGTCCACAGCTCCTTC CGGATGCAGGAAGATCAA	0,2 µM	63	653	Kozak *et al.*, 2009
sul1	sul1-F sul1-R	CGGCGTGGGCTACCTGAACG GCCGATCGCGTGAAGTTCCG	0,2 µM	66	433	Kozak *et al.*, 2009
sul2	sul2-L sul2-R	CGGCATCGTCAACATAACCT TGTGCGGATGAAGTCAGCTC	0,3 µM	66	721	Kozak *et al.*, 2009
sul3	sul3-GKa-F sul3-GKa-R	CAACGGAAGTGGGCGTTGTGGA GCTGCACCAATTCGCTGAACG	0,2 µM	66	244	Kozak *et al.*, 2009
dfrA7/*dfrA17*	DFRA7- F DFRA7-R	CAGAAAATGGCGTAATCG TCACCTTCAACCTCAACG	0,2 µM	50	345	Frech *et al.*, 2003
dfrA1	DFRA1-F DFRA1-R	GATATTCCATGGAGTGCCA ACCCTTTTGCCAGATTTG	0,2 µM	50	414	Frech *et al.*, 2003

The multiplex PCR were performed for: 1- bla_{TEM}, bla_{SHV}, bla_{CMY-2}, 2- tetB, tetC, tetD, 3- *aadA1, strA/strB, aac(3)IV,* 4- *sul1, sul2, sul3*.

(20/10 μg), cephalothin (30 μg), cefuroxime (30 μg), cefoperazone (75 μg), ceftazidime (30 μg), streptomycin (10 μg) neomycin (30 μg), amikacin (30 μg), gentamicin (10 μg) tetracycline (30 μg), doxycycline (30 μg), chloramphenicol (30 μg), nitrofurantoin (300 μg) trimethoprim/sulfamethoxazole (1.25/23.75 μg), norfloxacin (10 μg) (Becton Dickinson).

Identification of resistance genes. The presence of resistance genes to β-lactam antibiotics: bla_{TEM}, bla_{SHV}, and bla_{CMY-2}, tetracycline resistance genes: *tetA*, *tetB*, *tetC*, *tetD*, *tetM*, streptomycin: *strA*/*strB*, *aac(3)IV* and *aadA1*, sulfonamides: *sul1*, *sul2*, *sul3* and trimethoprim: *dfrA1* and *dfrA7*/*dfrA17* was detected by PCR. The primers sequences, their final concentration, PCR annealing temperatures, and the amplicon sizes are listed in Table I (Kozak *et al*., 2009; Ng *et al*., 2001; Frech *et al*., 2003). PCR assays in 25 μl final volume, contained: 2.5 μl 10 × PCR buffer, 2 mM $MgCl_2$, 0.25 mM dNTP (Promega), 1 μl Taq polymerase 2 U (Fermentas) and 1,5 μl DNA template (thermal lysates). Amplification program for the *tet* genes detection was as fallowed: initial denaturation at 94°C/5 min, 35 cycles: 94°C/1 min, annealing/30 s, 72°C/1 min and final extension 72°C/5 min, and for the remaining genes: 94°C/10 min, 30 cycles 94°C/30 s, annealing/1 min and 72°C/30 s, with final extension 72°C/10 min. Amplicons were separated electrophoretically in 1.5% agarose (1 × TBE), stained by ethidium bromide and documented (BioCapt). PCR products were also sequenced (Genomed) and compered to GenBank data base. The accession numbers were: bla_{TEM} GB:JQ416149.1, bla_{SHV} GB:AF148850.1, *tetA* GB:FN554766.1, *tetB* GB:HQ333262.1, *tetC* GB:EU751610.1, *aadA1* GB:JN596280.1, strA/strB FJ474091.2 *sul1* GB: JN003421.1, *sul2* GB:HQ018801.1, *sul3* GB: HQ875012.1, *dfrA1* GB:JN108887.1, *dfrA7*/*dfrA17* GB:JN108894.1.

Statistical analysis. Pearson's Chi squared test was used for determining the correlations between resistance genes prevalence and the source of *E. coli* origin with significance level set at $p < 0.05$ (statistical program R version 2.15.0) (Verzani, 2005).

Results

Susceptibility of *E. coli* strains was diversified both between different host species and within groups of animals (between young and adult swine and between groups of cattle from pastures and dairy cows from barn), whereas the outcomes from similar populations (of two groups of piglets, sows and cattle herds) were comparable. Sensitivity to all tested agents demonstrated only 3.6% of *E. coli* from pigs but 43.8% from cattle isolates. Resistance to streptomycin (88.3%), cotrimoxazole (78.8%), tetracycline (57.3%) and ampicillin (49.3%) was dominant among isolates from swine (Table II). In the total set of *E. coli* from swine, 81.0% of isolates were resistant to 3 or more antibiotics

Table II
Occurrence of antimicrobial resistance among *E. coli* isolated from group of swine and cattle.

Antibiotic	No. (%) of resistant isolates from:								
	Piglets I n = 59	Piglets II n = 51	Sows I n = 78	Sows II n = 86	Total in swine n = 274	Cattle pasture I n = 58	Cattle pasture II n = 50	Cattle barn n = 125	Total in cattle n = 233
Ampicillin	50 (84.7)	42 (82.4)	18 (23.1)	25 (29.1)	135 (49.3)	1 (1.7)	0 (0)	8 (6.4)	9 (3.9)
Amoxicillin/ Clavulanic Acid	4 (6.8)	0 (0.0)	0 (0.0)	0 (0.0)	4 (1.5)	2 (3.4)	0 (0)	11 (8.8)	13 (5.6)
Cephalothin	37 (62.7)	44 (86.3)	23 (29.5)	17 (19.8)	121 (44.2)	16 (27.6)	9 (18)	61 (48.8)	86 (36.9)
Cefuroxime	5 (8.5)	3 (5.9)	0 (0.0)	0 (0.0)	8 (2.9)	7 (12.1)	5 (10)	32 (25.6)	44 (18.9)
Cefoperazone	4 (6.8)	3 (5.9)	0 (0.0)	0 (0.0)	7 (2.6)	0 (0)	0 (0)	3 (2.4)	3 (1.3)
Ceftazidime	5 (8.5)	0 (0.0)	0 (0.0)	0 (0.0)	5 (1.8)	1 (1.7)	0 (0)	7 (5.6)	8 (3.4)
Streptomycin	57 (96.6)	51 (100)	64 (82.1)	70 (81.4)	242 (88.3)	3 (5.2)	0 (0)	5 (4.0)	8 (3.4)
Neomycin	2 (3.4)	0 (0.0)	1 (1.3)	2 (2.3)	5 (1.8)	0 (0)	0 (0)	1 (0.8)	1 (0.4)
Amikacin	0 (0.0)	0 (0.0)	2 (2.6)	0 (0.0)	2 (0.7)	1 (1.7)	1 (2)	10 (8.0)	12 (5.2)
Gentamicin	21 (35.6)	17 (33.3)	1 (1.3)	1 (1.2)	40 (14.6)	1 (1.7)	0 (0)	13 (10.4)	14 (6.0)
Tetracycline	47 (79.7)	33 (64.7)	41 (52.6)	36 (41.9)	157 (57.3)	0 (0)	0 (0)	3 (2.4)	3 (1.3)
Doxycycline	31 (52.5)	24 (47.1)	36 (46.2)	32 (37.2)	123 (44.9)	4 (6.9)	0 (0)	15 (12)	19 (8.2)
Chloramphenicol	34 (57.6)	21 (41.2)	30 (38.5)	26 (30.2)	111 (40.5)	3 (5.2)	3 (6)	6 (4.8)	12 (5.2)
Nitrofurantoin	8 (13.6)	2 (3.9)	5 (6.4)	0 (0)	15 (5.5)	2 (3.4)	0 (0)	16 (12.8)	18 (7.7)
Trimethoprim/ Sulfamethoxazole	56 (94.9)	47 (92.2)	50 (64.1)	63 (73.3)	216 (78.8)	1 (1.7)	1 (2)	2 (1.6)	4 (1.7)
Norfloxacin	1 (1.7)	0 (0.0)	1 (1.3)	0 (0.0)	2 (0.7)	0 (0)	3 (6)	0 (0)	3 (1.3)

Table III
Occurrence of multidrug resistance among *E. coli* isolated from groups of swine and cattle.

Multiresistance	Number (%) of isolates derived from								
	Pigs					Cattle			
	Piglets I n = 59	Piglets II n = 51	Sows I n = 78	Sows II n = 86	Total n = 274	Pasture I n = 58	Pasture II n = 50	Barn n = 125	Total n = 233
Resistant to 1 antibiotic	1 (1,7)	0 (0)	8 (10,3)	12 (14,0)	21 (7,7)	15 (25,9)	4 (8,0)	37 (29,6)	66 (28,3)
Resistant to 2 antibiotics	2 (3,4)	0 (0)	11 (14,1)	8 (9,3)	21 (7,7)	10 (17,2)	0 (0)	17 (13,6)	31 (13,3)
Resistant to 3 or more antibiotics	56 (94,9)	51 (100,0)	55 (70,5)	60 (69,8)	222 (81,0)	2 (3,4)	0 (0)	32 (25,6)	34 (14,6)
Sensitive	0 (0)	0 (0)	4 (5,1)	6 (7,0)	10 (3,6)	31 (53,4)	14 (28,0)	39 (31,2)	102 (43,8)

(Table III). Resistance to the majority of the tested antibiotics decreased with the age of the swine, and the ratio of resistance decrease (except for gentamicin) remained on a similar level.

Isolates from cattle demonstrated the highest resistance to cephalothin (36.9%), cefuroxime (18.9%), doxycycline (8.2%) and nitrofurantoin (7.7%) (Table II). Generally, lower resistance levels were observed in

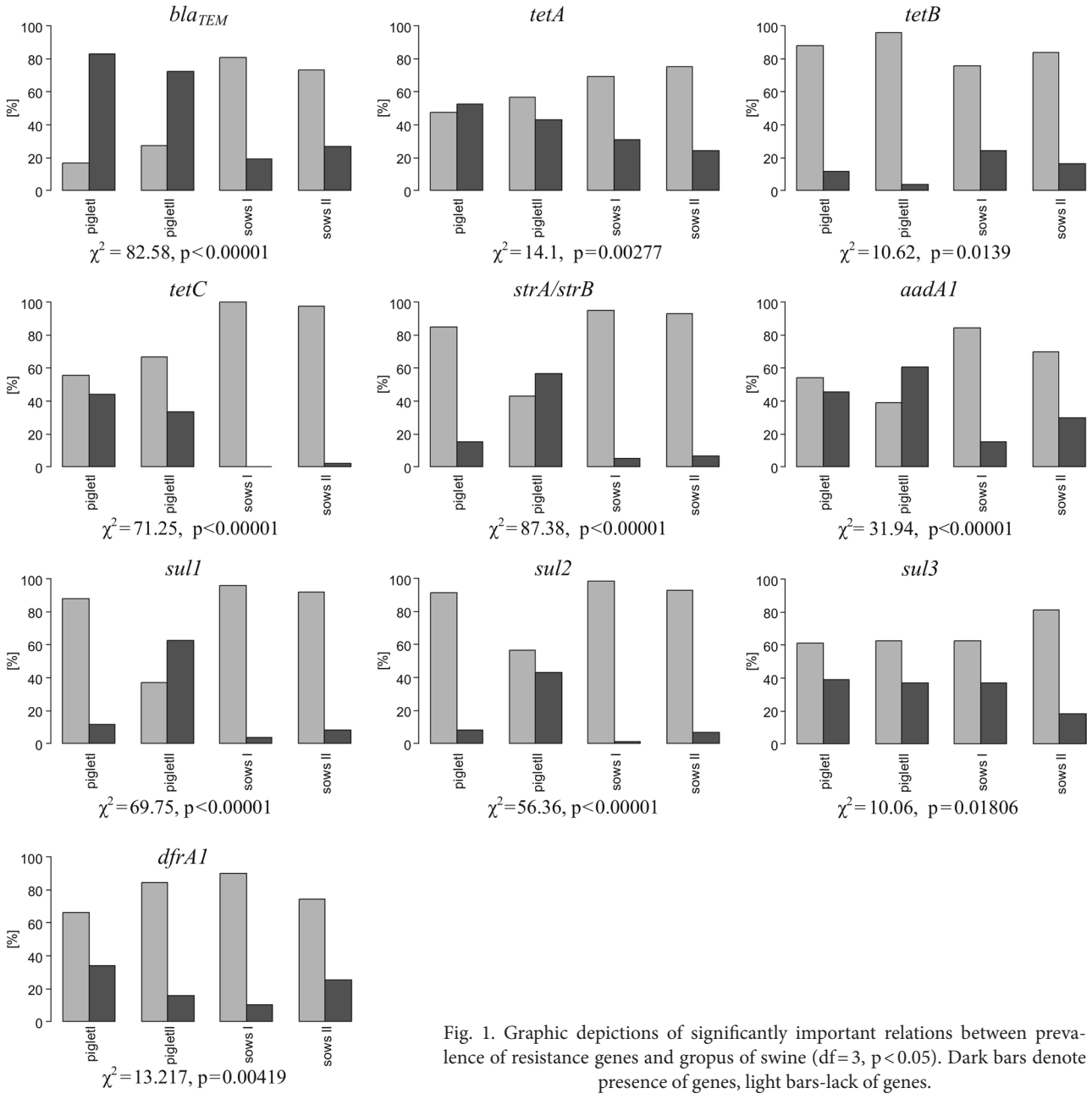

Fig. 1. Graphic depictions of significantly important relations between prevalence of resistance genes and gropus of swine (df = 3, p < 0.05). Dark bars denote presence of genes, light bars-lack of genes.

Table IV
Occurrence of resistance genes identified among *E. coli* isolated from group of swine and cattle.

Group of animal	Number (%) of gene-positive isolates corresponding to antibiotic resistance:													
	Ampicillin			Tetracycline			Streptomycin			Sulfonamide/Trimethoprim				
	Pheno type	bla_{TEM}	bla_{SHF}	Pheno type	*tetA*	*tetB*	*tetC*	Pheno type	*strA/strB*	*aadA*	Pheno type	*sul1*	*sul2*	*sul3*	*dfrA1*	*dfrA7/dfrA17*
Piglets I n = 59	50 (85)	49 (83)	0 (0)	37 (75)	31 (52)	7 (12)	26 (44)	57 (97)	7 (12)	27 (46)	56 (95)	9 (15)	5 (8.5)	23 (39)	20 (34)	2 (3)
Piglets II n = 51	42 (82)	37 (72)	0 (0)	33 (65)	22 (43)	2 (4)	17 (33)	51 (100)	32 (63)	31 (61)	47 (92)	29 57)	22 (43)	19 (37)	7 (14)	3 (6)
Sows I n = 78	18 (23)	15 (19)	0 (0)	41 (53)	24 (31)	19 (24)	0 (0)	64 82)	3 (4)	12 (15)	50 (64.1)	3 (4)	1 (1.3)	25 (32)	8 (10)	1 (1.3)
Sows II n = 86	25 (29)	23 (27)	0 (0)	36 (42)	21 (24)	14 (16)	2 (2.3)	70 (81)	7 (8)	26 (30)	63 (73)	6 (7)	6 (7)	14 (16)	21 (24)	3 (3.5)
Total in swine n = 274	135 (49)	124 (45)	0 (0)	157 (57)	98 (36)	42 (15)	45 (16)	242 (88)	49 (18)	96 (35)	216 (79)	47 (17)	34 (12)	81 (29)	56 (20)	9 (3.3)
Cattle pasture I n = 58	1 (2)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	3 (5.2)	0 (0)	0 (0)	1 (1.7)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Cattle pasture II n = 50	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (2)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Cattle barn n = 125	8 (6)	1 (0.8)	8 (6)	3 (2.4)	3 (2.4)	0 (0)	1 (0.8)	5 (4.0)	0 (0)	0 (0)	2 (1.6)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Total in cattle n = 233	9 (4)	1 (0.4)	8 (3)	3 (1.3)	3 (1.3)	0 (0)	1 (0.43)	8 (3.4)	0 (0)	0 (0)	4 (1.7)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)

None of bla_{CMY-2}, *tetD*, *tetM*, and *aac(3)IV* genes were detected.

isolates from cattle living on pastures than housed in a barn and multidrug resistance concerned mainly *E. coli* from dairy cows (Table III).

All the phenotypically resistant isolates were tested for the presence of the resistance genes. The most commonly identified gene in isolates from swine was bla_{TEM}, then *tetA*, *aadA1*, *sul3*, *dfrA1*, *strA*/*strB*, *sul1*, *tetC, tetB*, *sul2* and *dfrA7*/*dfrA17* (Table IV), and differences in the prevalence of resistance genes between *E. coli* from piglets and sows were observed. The results of Pearson's Chi squared tests indicated a significant relation between the prevalence of resistance genes in commensal *E. coli* from swine and the age group of these animals (Fig. 1). The distribution of bla_{TEM}, *tetA* and *tetC* genes in *E. coli* decreased with the age of the swine, but for *tetB* gene the decrease occurred separately in isolates derived from young and adult pigs. For the *strA*/*strB, aadA1*, *sul1* and *sul2* genes the prevalence increased in *E. coli* from piglets, but decreased in sows (Fig. 1). The relation of the prevalence of *sul3* and *dfrA1* genes was maintained at a similar levels in isolates from the analyzed groups of swine. For *dfrA7*/*dfrA*17 gene differences in occurring were not statistically significant ($p = 0.5557$). Predominant resistance gene profiles were different for isolates from various groups of swine. More complex resistance gene profiles (consisting of 2 to 6 genes) were found in strains from piglets., whereas simpler profiles occurred in strains from sows. The most commonly observed resistance genotype patterns are placed in Table V. None of the examined genes were found in *E. coli* from beef cows feeding on pastures, but 8 bla_{SHV}, 1 bla_{TEM}, 2 *tetA*, and 1 combination of *tetA*+*tetC* genes were identified in *E. coli* from dairy cows housed in the barn.

Discussion

The results of research concerning relations between antibiotic input and resistance development vary in terms of geographic location, bacterial species and antimicrobials tested (Jacob *et al*., 2008). Few studies have examined conventional and organic breeding. In conventional production on a large scale, antibiotic usage is a part of the production system, not only in veterinary practice, but also as a prophylactic measure, on account of numerous illnesses. Gastrointestinal and respiratory infections are very common, especially in piglets during their weaning period, and *E. coli* is one of the pathogens (Aarestrup *et al*., 2008). Cattle are less sensitive to illnesses, thus there is no need to administer preventive antibiotic supplements and it is mainly dairy cows with mastitis that require treatment.

The presented data demonstrate a relationship between selective pressure of antibiotic and the emergence of resistance. Antibiotic resistance patterns

Table V
The most commonly detected gene patterns in *E. coli* isolates from groups of swine.

Piglets I	Piglets II	Sows I	Sows II
bla_{TEM}, *tetA/B/C*	bla_{TEM},*tetC*, *strA/B*, *aadA1*, *sul1*, *sul3*	*tetB*	bla_{TEM}
bla_{TEM}, *tetA*, *tetC*	*tetA*, *strA/B*, *aadA1*, *sul1/2*, *dfrA1*	*sul3*	*tetA*
bla_{TEM}, *tetA/tetC*, *sul3*	bla_{TEM}, *tetC*, *sul3*	*tetA*	*tetB*
bla_{TEM}, *tetB/C*, *strA/B*, *aadA1*, *sul3*	bla_{TEM}, *sul2*	*tetA/tetB*, *sul1/3*	*aadA1*
bla_{TEM}, *tetC aadA1*, *sul1*, *sul2*, *dfrA1*	bla_{TEM}, *strA/B*, *aadA1*, *sul1/2/3*	bla_{TEM}, *tetA/tetB*, *sul1/3*	*tetA/tetB*, *dfrA1*

differed between *E. coli* from different hosts, from very high resistance levels in swine isolates to low frequency of resistance in *E. coli* from cattle. The occurrence of high resistance to co-trimoxazole and ampicillin in piglets was the consequence of antibiotic supply in their post-weaning period. In older pigs (sows I, II) with no antibiotic pressure, resistance to these agents decreased: slightly to co-trimazol (93.6% in piglets vs. 68.9% in sows) and significantly to ampicillin (83.6% in piglets vs. 26.2% in sows) but still remained. Very high resistance levels and multiple resistances up to 9 agents were observed, with great proportion for aminoglycosides (streptomycin) and tetracyclines. These antibiotics are used in veterinary treatment, but the subject animals were not treated with these agents. That clearly indicates the accumulation of resistance gene cassettes and dissemination in population. The presented data show that *E. coli* from pigs share a similar gene pool. Additionally, great dynamics of their appearance was found, which reflects different genetic patterns, beginning with a single resistance gene detected in isolates from sows up to complex patterns containing 6 genes in isolates from piglets. The prevalence of resistance genes corresponding to β-lactam antibiotics (bla_{TEM}) and tetracyclines (*tetA* and *tetC*) decreased along with the age of the swine. Resistance to streptomycin stayed on high level, but the dissemination of corresponding resistance genes altered. The increase in frequency of *strA*/*strB* and *aadA1* gene was observed in strains from piglets during antibiotic administration (medical preventive program), whereas lower dissemination was observed in isolates from sows. The high resistance to co-trimazole was found in *E. coli* from all analyzed groups of swine, and the prevalence of resistance genes *dfrA1* and *sul3* was also identified. However, the frequency of *sul1* and *sul2* resistance genes increased in piglets and decreased in sows.

The predominant resistant genotype revealed in this research in *E. coli* from swine (bla_{TEM}, *tetA, aadA1, sul3, dfrA1*) was different from that observed by Kozak *et al.* in Canada (2009), where the most commonly detected genes were, in order of decreasing prevalence: *tetB*, *aadA1*, *strA*/*strB*, *tetA*. Also dissimilar results (*tetD*, *tetA*, *dfrA1*, *aadA1*, *strA*/*strB*) were received by Frye *et al.* (2011), where *E. coli* from pigs in Georgia (USA) was examined. In this study the tetracycline resistance genes *tetA* and *tetB* were the most frequently identified in *E. coli* from adult swine and cattle and this stayed in agreement with the outcomes of other research referring to the intestinal bacteria of food animals, pigs and cattle (Bryan *et al.*, 2004; Sengeløv *et al.*, 2003).

The research revealed that the resistance prevalence in *E. coli* from swine differed from the resistance prevalence found in isolates from cattle. Apart from cross-resistance to cephalosporins, low resistance (under 10%) to the remaining antibiotics was observed. These low resistance rates illustrate the lack or only occasional occurrence of antibiotic pressure in these populations. In comparison to resistance genes prevalence in isolates from swine, very few resistance genes were identified in isolates from cattle. In *E. coli* from pigs, the resistance gene corresponding to resistance to β-lactam antibiotics was bla_{TEM}, whereas in isolates from cattle it was gene bla_{SHV}. Contrary to these data, a few studies revealed high, multiple resistance with complex genetic patterns in isolates derived from cattle either from conventional farming or with the treatment applied (Guerra *et al.*, 2003; Karczmarczyk *et al.*, 2011). In the presented paper, phenotypic resistance and resistance genes were identified mainly in isolates from cows housed in the barn. This can be an effect of the dissemination of resistant strains from humans to animals. Several studies indicated that antimicrobial resistance rates are higher among animals with human exposure than on isolated areas with little contact with humans (Kozak *et al.*, 2009; Skurnik *et al.*, 2006). The presented research indicates that *E. coli* strains originating from herds of cows grazed on a pasture without any antibiotic pressure do not constitute meaningful reservoir of resistance genes.

Antibiotic administration in food-producing animals has resulted in greater breeding success in one aspect, but it contributes to resistance development, therefore these animals constitute the reservoir for antimicrobial resistance genes. The presented data confirm a link between exposure to antibiotics and resistance development. They indicate that once generated resistance can fluctuate but persists in a population.

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