CODA-CERVA

VETERINARY AND AGROCHEMICAL RESEARCH CENTRE

Report: antimicrobial resistance in commensal *E. coli* from poultry, pigs, cows and veal calves. 2012

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1 Introduction

Commensal *E. coli* are regarded as general indicators for resistance amongst Gram negative bacteria. They have the advantage of being present in nearly all animal species and in large numbers. As such they can be isolated from an animal at almost every sampling occasion. Because they are continuously present, they can be used to follow up resistance evolution in time.

Moreover they have been studied frequently in other countries so they are useful in comparing geographic distribution of antimicrobial resistances. The genetic background or resistance in this species is also quite well known allowing a scientific interpretation of the resistance data.

This is the report, the second year of surveillance of antimicrobial resistance in commensal *E. coli* from animals in Belgium is presented. The surveillance programme is conceived to follow up trends in prevalence of resistance at the national level. The third sample of 2013 will allow us to detect eventual trends in evolution of antimicrobial resistance. This is important seen measures are being prepared to reduce antimicrobial usage and the effect of the measures can then be compared to this baseline set.

2 Materials and Methods

2.1 Sampling

Samples from faecal material were taken from 4 animal categories: broiler chickens, pigs, bovines (for meat production) and veal calves. Samples were taken by samplers of the Belgian Food Agency.

2.1.1 Poultry

Caecal content of broiler chickens was taken at slaughter together with the samples in the framework of *Salmonella* control programme. Caeca from 10 animals were collected and pooled. One sample originated from one farm.

2.1.2 Pig

Pooled fresh faecal material of at least ten animals of approximately 6 months old was collected from slaughter pigs at the abattoir. One sample originated from one farm.

2.1.3 Bovines

Pooled fresh faecal material was collected from the floor of barns harbouring bovines for meat production of less than 7 months of age. One sample originated from one farm.

2.1.4 Veal calves

Pooled fresh faecal material was collected at the abattoir from veal calves of less than 7 months of age.

2.2 Isolation and identification

Faecal material was inoculated and *E. coli* was identified at DGZ or ARSIA.

At DGZ faecal material was inoculated on McConkey agar and incubated at 37°C for 18 to 24 hours. Suspected colonies (pink, lactose positive) were inoculated on Kligler and indol medium and incubated at 37°C for 18 to 24 hours. When the test outcome was positive for *E. coli* a colony from the Kligler medium was inoculated on Mac Conckey agar, incubated at 37°C for 18-24 hours and sent to CODA-CERVA.

At ARSIA, faecal material was inoculated on Gassner medium and incubated at 37°C for 18 to 24 hours. Suspected colonies were purified on Columbia agar supplemented with 5% sheep blood. Identification was done by the OPNG test, Ureum test and indol test. Confirmed *E. coli* were sent to CODA-CERVA.

2.3 Susceptibility testing

Strains were sent to the national reference laboratory (CODA-CERVA) for susceptibility testing. Upon arrival, the strain was purified on Columbia agar with 5% sheep blood and susceptibility was tested using a micro broth dilution method (Trek Diagnostics). To this end, 1 to 3 colonies were suspended in sterile distilled water to an optical density of 0.5 McFarland. Ten microliter of this suspension is inoculated in 11ml cation adjusted Mueller Hinton broth with TES buffer.

Fifty microliter of the Mueller-Hinton broth with bacteria was brought on a micro-titer plate with the antimicrobials lyophilised, the EUMSV2 plate as produced by Trek Diagnostics, using the auto-inoculating system of Trek Diagnostics. The concentrations tested are indicated in table 1 (grey zones are the concentrations tested).

Plates were incubated 18-24 hours at 35°C and read. The Minimal Inhibitory Concentration (MIC) was defined as the lowest concentration by which no visible growth could be detected. MICs were semi-automatically recorded by the Trek Vision system using the SWIN software. Results were automatically exported to an Excel file.

Table 1 shows the antimicrobials tested and their abbreviations. Concentrations tested are shown in table 2.

2.4 Analysis of data

Data from the Excel file generated by the software of the semi-automated susceptibility equipment (sensivision, Trek Diagnostics) and merged to the administrative date from the LIMS system at CODA. These files were validated for consistency. The excel file was then imported into an Access file in which the number of strains having an MIC for a certain antibiotic were calculated. These data were set in a table that was subsequently exported to an Excel file. The data were interpreted for susceptibility using breakpoints based on the EUCAST ECOFFs or as defined by the EU reference laboratory on antimicrobial resistance (DTU) are indicated.

The number of resistant strains was counted and resistance percentages were calculated. Exact confidence intervals for the binomial distribution were calculated using a visual basic application in Excel. A 95% symmetrical two-sided confidence interval was used with p=0.025. The lower and upper bound of confidence interval for the population proportion was calculated.

Based on the Pearsons chi-square test, and where appropriate the Fischer exact test, significance of the differences were calculated. This was also done to compare the data from 2011 with those from 2012.

Multi-resistance was determined by transforming the MIC data into resistant (R) and susceptible (S). Number of antimicrobials to which a strain was resistant to was counted and cumulative percentages were calculated. The modal number of antimicrobials to which 50% of the strains was resistant was calculated. Graphical representations were prepared in excel.

3 Results

Results are shown in tables 2 to 10 and figures 1 to 8.

3.1 Poultry

Susceptibility of commensal *E. coli* towards the different antimicrobials tested is shown in table 2. A total of 324 strains were tested. Highest resistance was seen against streptomycin followed by ampicillin, sulphonamide, ciprofloxacin and nalidixic acid resistance, closely followed by tetracycline and trimethoprim resistance. Nearly half of the strains were resistant to chloramphenicol. Over a quarter of the strains was resistant to extended spectrum cephalosporins. Resistance against the aminoglycosides kanamycin and gentamicin remained low, and the resistance against florfenicol and colistin remained below 5%.

Ciprofloxacin and nalidixic acid are both antimicrobials from a same class and likewise, resistance is frequently cross-resistance. The extreme high prevalence of fluoroquinolone resistance is worrisome. It should be noted that this resistance is mainly low level resistance, and that approximately 55 strains (17%, CI: 13,1—22) demonstrated high level resistance. We see more resistance against ciprofloxacin, which indicates the presence of mobile fluoroquinolone resistance, without mutational resistance. This resistance can be mediated by *qnr* (DNA protection), *qepA* (efflux) or *aac*(6')-Ib-cr genes (aminoglycoside acetyl transferase showing cross resistance with several aminoglycosides). There are actually no data available on the prevalence of plasmid mediated fluoroquinolone resistance. It should be noted that this type of resistance can be masked by the presence of mutational resistance.

All the florfenicol resistant strains showed cross-resistance to chloramphenicol. It should be noted that chloramphenicol resistance was significantly higher than in 2011 (nearly double). It remains to be seen whether this is a trend since the current data do not allow trend analysis. The reason for this high level of resistance remains unclear seen chloramphenicol is not used anymore for over 20 years. Since there is no concomitant resistance to florfenicol, this resistance is mainly due to the “old” gene, still circulating. Florfenicol resistance is associated with multi-resistance (at least 8 antibiotics and up to 14, which is resistant to all antibiotics tested), similarly as in *Salmonella*. In the latter, the *flo* gene, encoding florfenicol resistance is associated to an insertion sequence (mini-transposon) that preferentially inserts in the 5’ part of integrons that are present in the *Salmonella* genomic island, encoding for the typical penta-resistance. This island has never been demonstrated in *E. coli*, but integrons have been detected on many occasions. This may explain its association with multi-resistance.

Resistance to the cephalosporins cefotaxime and ceftazidime is between 25 and 30%. Also these percentages are significantly higher than last year. Few ceftazidime resistant strains were not resistant to cefotaxime, as expected, while some more strains were cefotaxime resistant and not ceftazidime resistance. These are suspected to be ESBL carrying strains. However, confirmatory testing is necessary to determine the full phenotype. The strains are clearly multi-resistant with all strains resistant to more than 7 antimicrobials. Compared to last year, there is a shift towards more resistance to both antibiotics.

Contrary to streptomycin, there is little resistance to the other aminoglycosides tested (gentamicin and kanamycin). Colistin resistance remains negligible. The strains are however associated with multi-resistance (13 strains are resistant to more than 10 antibiotics, 2 to nine antimicrobials and 1 to 4).

Resistance to sulphonamides and trimethoprim is also substantial. Resistance to the combination (as determined by resistance to both components) is evident in 69.1% of the strains (CI 63.8-74), wich is also substantial.

Only 4.6% of the strains remained fully susceptible, which is lower than in 2011. A little more than 50% of the strains was resistant to at least 7 different antimicrobials, which is 2 more than last year. However, cross-resistances should be taken into account. All nalidixic acid resistant strains are also resistant to ciprofloxacin, and strains resistant to cephalosporins are also resistant to ampicillin. Similarly, but of little influence is that florfenicol resistant strains are chloramphenicol resistant.

One strain was resistant to all antimicrobials tested and four strains remained susceptible to only one antibiotic. In three cases this was colistin and one case this was florfenicol. The four strains resistant to twelve antibiotics remained susceptible to colistin (2 cases), chloramphenicol (1 case), florphenicol (2 cases), cefotaxime (1case) and gentamicin (1case) and streptomycine (1case).

3.2 Pigs

Susceptibility of commensal *E. coli* towards the different antimicrobials tested is shown in table 4. For four antimicrobials, resistance was higher than 50% (sulphonamides, streptomycin tetracycline and trimethoprim). The combination sulphonamide-trimethoprim was determined and 49.5% (CI 42.6-56) of the strains was resistant against the combination, wich is only marginally lower than for the separate components, indicating that most strains are resistant to both compared to the single components separately. Resistance against 6 of the antibiotics tested remained below 5%, of which notably colistin, an antibiotic largely used in the treatment of diarrhoea. The two colistin resistant strains were highly multi-resistance with one strain being resistant to 8 antibiotics and the other one resistant to 7 antibiotics. Also cephalosporin resistance remains low. Resistance to chloramphenicol remains relatively high with nearly 30% if the strains being resistant. Also here, since florfenicol resistance is below 5%, the majority of this resistance is to be explained by the presence of the “old” resistance gene and not the *flo* gene causing resistance to both chloramphenicol and florfenicol. Resistance against aminoglycoside, except for streptomycin, remains low.

Ciprofloxacin resistance is higher than nalidixic acid resistance. Also here the presence of plasmid mediated quinolone resistance (PMQR) is suspected in 12 strains.

Less than a quarter of the strains remained fully susceptible. Half of the commensal *E. coli* from pigs were resistant to at least 3 antimicrobials. Three strains were resistant to as much as 11 antimicrobials. They had the same susceptibility profile and remained susceptible to colistin, gentamicin and florfenicol.

Comparing the data from 2011 and 2013, using the chi square test, there was only a difference noted for streptomycin for which more resistance was detected. There were no other differences in resistance prevalence between 2011 and 2012. This does not exclude that there are eventual trends but to determine this, at least 3 sampling points are necessary and other statistical tests should be used at that time.

3.3 Bovines

The highest prevalence of resistance in bovine commensal *E. coli* is against sulphonamides, tetracycline and ampicillin with resistance percentages over 30%. Just below 30% resistance is trimethoprim resistance. Approximately one fifth of the strains is resistant against chloramphenicol and ciprofloxacin. Other resistances are less than 15% and for most less than 10%. The combined resistance against trimethoprim-sulphonamides, as calculated from the combine resistance against the two components is 28% (CI: 21.5-35). This is nearly the same as trimethoprim resistance indicating that nearly all trimethoprim resistant strains are resistant to sulphonamides.

Cefotaxime resistant strains were, except for two strains also resistant to ceftazidime. This indicates the presence of AmpC genes, however, confirmatory tests should be executed to confirm this. These strains were also highly multi-resistant compared to than the other strains with resistances to at least 3 different antibiotics, and the majority resistant against more than 9 antibiotics.

Also florfenicol resistance was associated with a high degree of multi-resistance. Similarly as in poultry and pigs, the” old” chloramphenicol resistance gene is still largely present.

In 2012 5 colistin resistant strains were detected, and these were resistant to at least 6 antibiotics.

Also here, ciprofloxacin resistance was higher than nalidixic acid resistance, indicating the presence of plasmid mediated quinolone resistance.

In 2012 less than half of the strains are fully susceptible to all antimicrobials tested. In contrast, two strains are resistant to 12 different antimicrobials. Those two strains were only susceptible to colistin and florfenicol. The strain, resistant to 10 antimicrobials was additionally susceptible to kanamycin and ceftazidime.

Compared to 2011 and using the chi square test, significant higher resistances were found against ciprofloxacin and tetracycline. Other resistances were in general also higher, but not significant. The third sampling will indicate wither this is a trend.

3.4 Veal calves

Resistance in commensal *E. coli* from veal calves is remarkably higher than in bovines. Against ampicillin, sulphonamides, tetracyclines and trimethoprim, resistance was approximately 70% and higher. Streptomycin resistance was only a bit lower and resistance against chloramphenicol and ciprofloxacin is more than 40%. Kanamycin resistance is approx. 30%. Resistance against other bacteria is 10% or lower. The combined resistance against trimethoprim-sulphonamides, as calculated from the combined resistance against the two components is 67,4% (CI: 60,1-74). This is nearly the same as trimethoprim resistance indicating that nearly all trimethoprim resistant strains are resistant to sulphonamides.

While last year, no cephalosporin resistance has been found, this year about 10% of the strains are resistant. As seen in the in *E. coli* from other animal species, these strains are also highly multi resistant, ranging from resistance against 7 antibiotics to 13. Most strains were resistant to more than 10 antimicrobials.

Colistin resistance was also associated with multi-resistance, though there was also one strain, only resistant to colistin.

As for other animal species, also here chloramphenicol resistance is much higher compared to florfenicol.

Only a bit more than 10% of the strains was fully susceptible. Fifty per cent of the strains was resistant to at least 6 antibiotics. This indicates a high level of multi-resistance among the resistant strains. Three strains are resistant up to 12 antibiotics and one strain was resistant to as much as 13 antibiotics.

Compared to 2011, there were no significant differences, though it should be noted that in 2011, only few strains were tested, with as a consequence large confidence intervals, which do not allow detecting more discrete changes. Anyhow, for trends, a third sampling year is necessary.

3.5 Comparison between animal species

Clearly, commensal *E. coli* from poultry and veal calves are the most resistant strains. Few strains remain fully susceptible. Multi-resistance could be as high as to 14 and 13 antibiotics for poultry and veal calves respectively. For pigs the situation was less dramatic with more than one fifth of the strains remaining fully susceptible and for bovines a bit less than half of the strains remained fully susceptible. The multi-resistance in bovines is quite equally distributed, also with two strains resistant to as much as 13 antibiotics, indicating that highly multi resistant strains are present in the population.

3.6 Conclusions

In conclusion, though we cannot determine yet whether it is a trend since the surveillance is only running for two years, poultry strains seem to increase in resistance, whit nearly all strains being resistant to one antibiotic and one strain resistant to all antimicrobials tested. Also multi-resistance has increased substantially, from 5 to 7 antibiotics as a median. Poultry strains were also the most resistant strains compared to the *E. coli* strains from other animal species. While last year, few strains were tested, we have now more accurate data on resistance prevalence in veal calves.

There are indications that PMQR is increasing. Since much of this resistance may be masked by mutational resistance, it warrants further follow up.

Typically, resistance against streptomycin is much higher than against the other aminoglycosides tested.

Cephalosporin resistance is establishing itself in the different populations, though mainly in poultry.

Finally, sampling of pigs and veal calves has changed. Now animals were all sampled at slaughter age. The influence of this is not clear.

Table 1. List of abbreviations

Abbreviation	
AMP	Ampicillin
CHL	Chloramphenicol
CIP	Ciprofloxacin
COL	Colistin
FFN	Florphenicol
FOT	Cefotaxime
GEN	Gentamicin
KAN	Kanamycin
NAL	Nalidxic acid
SMX	Sulphonamide
STr	Streptomycin
TAZ	Ceftazidime
TET	Tetracycline
TMP	Trimethoprim

Table 2. Antibiotic resistance in commensal *E. coli* from poultry.

Concentration	AMP	CHL	CIP	COL	FFN	FOT	GEN	KAN	NAL	SMX	Str	TAZ	TET	TMP
≤0.008	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0.015	0	0	51	0	0	0	0	0	0	0	0	0	0	0
0.03	0	0	14	0	0	0	0	0	0	0	0	0	0	0
0.06	0	0	4	0	0	212	0	0	0	0	0	0	0	0
0.12	0	0	35	0	0	14	0	0	0	0	0	0	0	0
0.25	0	0	100	0	0	5	1	0	0	0	0	224	0	0
0.5	0	0	35	0	0	8	141	0	0	0	0	18	0	86
1	0	0	15	0	0	6	152	0	0	0	0	23	15	10
2	31	2	15	309	1	12	11	0	0	0	0	12	78	3
4	28	57	10	6	124	20	6	264	62	0	8	8	4	2
8	4	101	30	9	162	47	3	14	6	3	37	14	2	2
16	0	18	15	0	25	0	9	2	3	24	13	12	5	1
32	2	38	0	0	5	0	0	3	10	27	26	13	11	3
64	259	28	0	0	5	0	1	0	75	9	33	0	65	216
128	0	80	0	0	2	0	0	5	168	2	33	0	144	0
256	0	0	0	0	0	0	0	36	0	1	174	0	0	0
512	0	0	0	0	0	0	0	0	0	1	0	0	0	0
1024	0	0	0	0	0	0	0	0	0	2	0	0	0	0
2048	0	0	0	0	0	0	0	0	0	255	0	0	0	0
Total	324	324	324	324	324	324	324	324	324	324	324	324	324	323
NR	261	146	259	15	12	93	19	46	253	261	266	82	225	224
%R	80,6	45,1	79,9	4,6	3,7	28,7	5,9	14,2	78,1	80,6	82,1	25,3	69,4	69,3
CI	75,8-85	39,6-51	75,2-84	2,6-8	1,9-6	23,8-34	3,6-9	10,6-18	73,2-82	75,8-85	77,5-86	20,7-30	64,1-74	63,8-74

3. Multi-resistance in commensal *E. coli* from poultry.

Number of antimicrobials	Number of strains	Cumulative number of strains	Cumulative %
0	15	15	4,6296
1	14	29	8,9506
2	8	37	11,42
3	14	51	15,741
4	26	77	23,765
5	17	94	29,012
6	29	123	37,963
7	53	176	54,321
8	51	227	70,062
9	45	272	83,951
10	31	303	93,519
11	12	315	97,222
12	4	319	98,457
13	4	323	99,691
14	1	324	100

Figure 1. Number of strains with multi-resistances from poultry

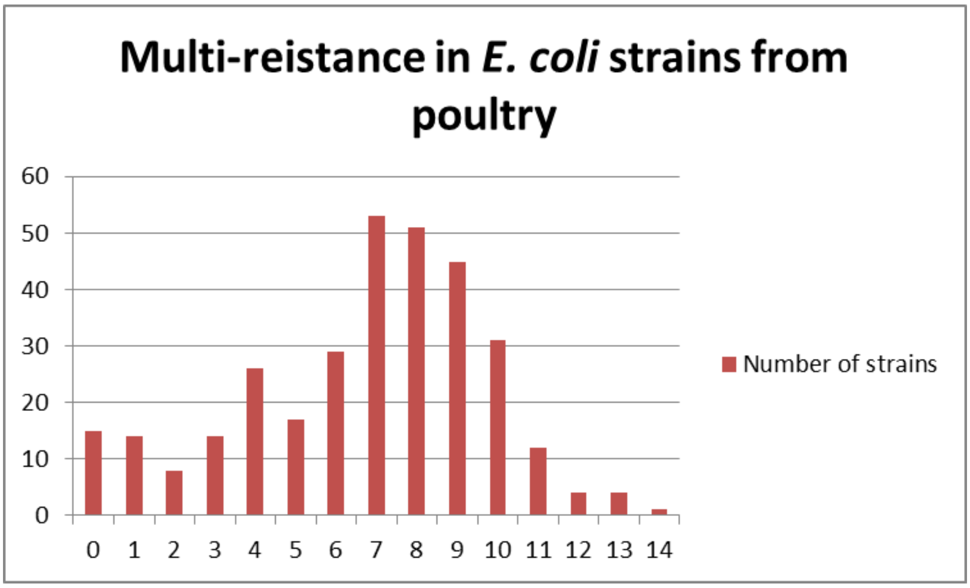

Figure 2. Cumulative percentage of multi-resistances from poultry

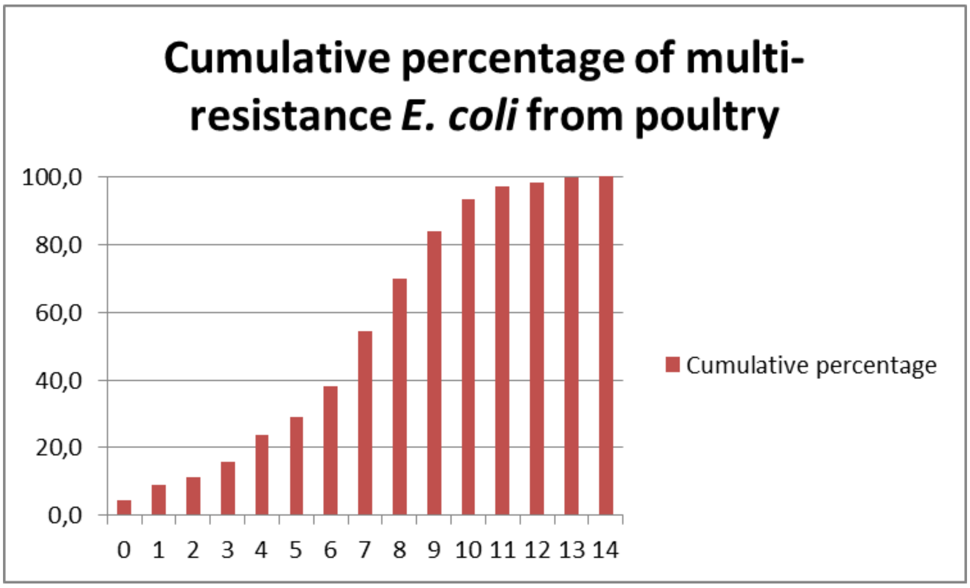

Table 4. Antimicrobial resistance in commensal *E. coli* from pigs

Concentration	AMP	CHL	CIP	COL	FFN	FOT	GEN	KAN	NAL	SMX	Str	TAZ	TET	TMP
<=0.008	0	0	8	0	0	0	0	0	0	0	0	0	0	0
0.015	0	0	137	0	0	0	0	0	0	0	0	0	0	0
0.03	0	0	32	0	0	0	0	0	0	0	0	0	0	0
0.06	0	0	9	0	0	195	0	0	0	0	0	0	0	0
0.12	0	0	5	0	0	11	0	0	0	0	0	0	0	0
0.25	0	0	15	0	0	0	1	0	0	0	0	194	0	0
0.5	2	0	2	0	0	1	81	0	0	0	0	10	0	94
1	4	0	0	0	0	2	118	0	0	0	0	3	20	3
2	50	2	0	210	5	1	10	0	0	0	0	3	56	2
4	50	36	0	2	72	2	0	200	178	0	15	1	8	1
8	4	104	4	0	101	0	0	3	5	14	60	1	2	0
16	2	8	0	0	24	0	0	2	4	36	26	0	3	1
32	1	23	0	0	2	0	0	0	5	24	21	0	7	2
64	99	18	0	0	2	0	2	1	4	14	21	0	42	109
128	0	21	0	0	6	0	0	0	16	3	30	0	74	0
256	0	0	0	0	0	0	0	6	0	2	39	0	0	0
512	0	0	0	0	0	0	0	0	0	1	0	0	0	0
1024	0	0	0	0	0	0	0	0	0	1	0	0	0	0
2048	0	0	0	0	0	0	0	0	0	117	0	0	0	0
Total	212	212	212	212	212	212	212	212	212	212	212	212	212	212
NR	102	62	35	2	10	6	2	9	25	124	111	8	126	113
%R	48,1	29,2	16,5	0,9	4,7	2,8	0,9	4,2	11,8	58,5	52,4	3,8	59,4	53,3
CI	41,2-55	23,2-36	11,8-22	0,1-3	2,3-9	1-6	0,1-3	2-8	7,8-17	51,5-65	54,4-59	1,6-7	52,6-66	55,8-69

Table 6. Multi-resistance in commensal *E. coli* from pigs

Number of antimicrobials	Number of strains	Cumulative number of strains	Cumulative %
0	47	47	22,2
1	24	71	33,5
2	17	88	41,5
3	18	106	50,0
4	21	127	59,9
5	33	160	75,5
6	18	178	84,0
7	18	196	92,5
8	10	206	97,2
9	3	209	98,6
10	0	209	98,6
11	3	212	100,0
12	0	212	100,0
13	0	212	100,0
14	0	212	100,0

Figure 3. Number of strains with multi-resistances from pigs

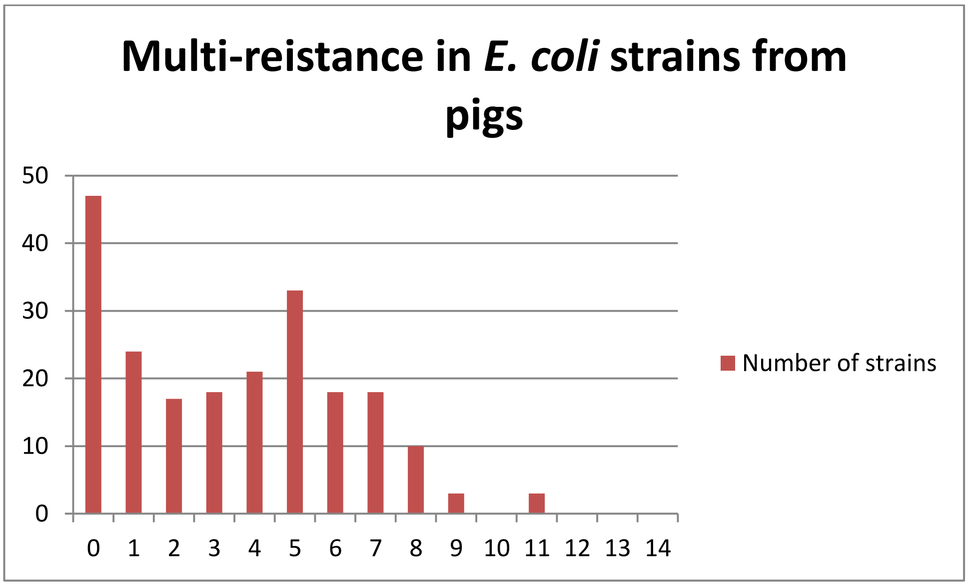

Figure 4. Cumulative percentage of multi-resistances from pigs

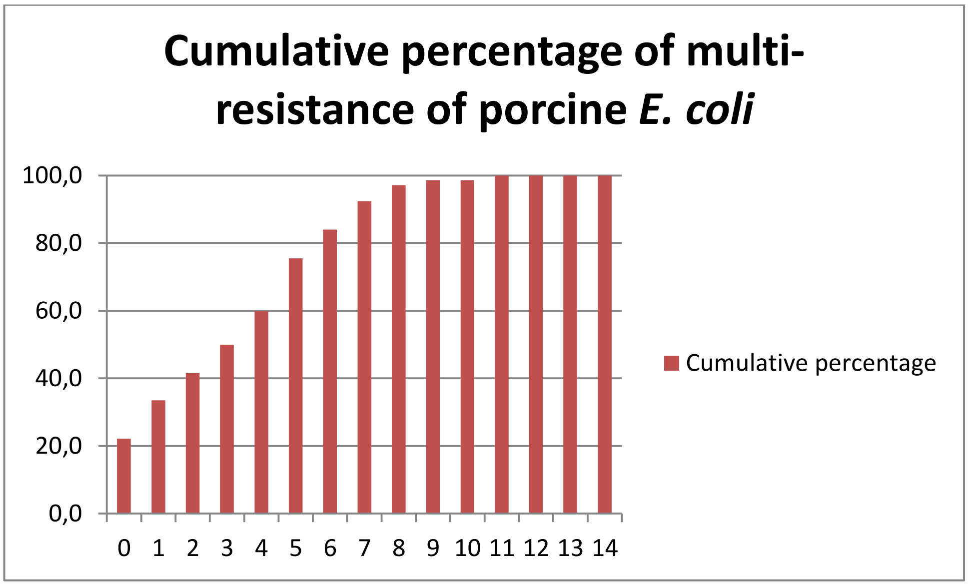

Table 7. Antibiotic resistance in commensal *E. coli* from bovines

Concentration	AMP	CHL	CIP	COL	FFN	FOT	GEN	KAN	NAL	SMX	Str	TAZ	TET	TMP
<=0.008	0	0	7	0	0	0	0	0	0	0	0	0	0	0
0.015	0	0	115	0	0	0	0	0	0	0	0	0	0	0
0.03	0	0	17	0	0	0	0	0	0	0	0	0	0	0
0.06	0	0	4	0	0	153	0	0	0	0	0	0	0	0
0.12	0	0	7	0	0	9	0	0	0	0	0	0	0	0
0.25	0	0	13	0	0	2	2	0	0	0	0	155	0	0
0.5	0	0	3	0	0	2	84	0	0	0	0	7	0	117
1	3	0	0	0	0	2	82	0	0	0	0	6	24	8
2	47	7	0	170	8	2	0	0	0	0	0	2	79	0
4	57	43	2	4	69	0	1	147	136	0	24	2	5	0
8	6	87	4	1	82	5	3	5	5	7	79	2	4	1
16	1	7	3	0	3	0	2	1	4	42	7	1	5	0
32	1	2	0	0	0	0	0	2	3	32	7	0	7	2
64	60	3	0	0	4	0	1	1	8	20	9	0	16	47
128	0	26	0	0	9	0	0	1	19	4	17	0	35	0
256	0	0	0	0	0	0	0	18	0	2	32	0	0	0
512	0	0	0	0	0	0	0	0	0	2	0	0	0	0
1024	0	0	0	0	0	0	0	0	0	8	0	0	0	0
2048	0	0	0	0	0	0	0	0	0	58	0	0	0	0
Total	175	175	175	175	175	175	175	175	175	175	175	175	175	175
NR	62	31	36	5	13	11	7	23	30	74	65	13	63	50
%R	35,4	17,7	20,6	2,9	7,4	6,3	4,0	13,1	17,1	42,3	37,1	7,4	36,0	28,6
CI	28,4-43	12,4-24	18,4-27	0,9-7	4-12	3,2-11	1,6-8	8,5-19	11,9-24	34,9-52	30-45	4-12	28,9-44	22-36

Table 8. Multi-resistance in commensal *E. coli* from bovines

Number of antimicrobials	Number of strains	Cumulative number of strains	Cumulative %
0	82	82	46,9
1	17	99	56,6
2	13	112	64,0
3	7	119	68,0
4	3	122	69,7
5	10	132	75,4
6	13	145	82,9
7	5	150	85,7
8	7	157	89,7
9	6	163	93,1
10	4	167	95,4
11	4	171	97,7
12	2	173	98,9
13	2	175	100,0
14	0	175	100,0

Figure 5. Number of strains with multi-resistances from bovines

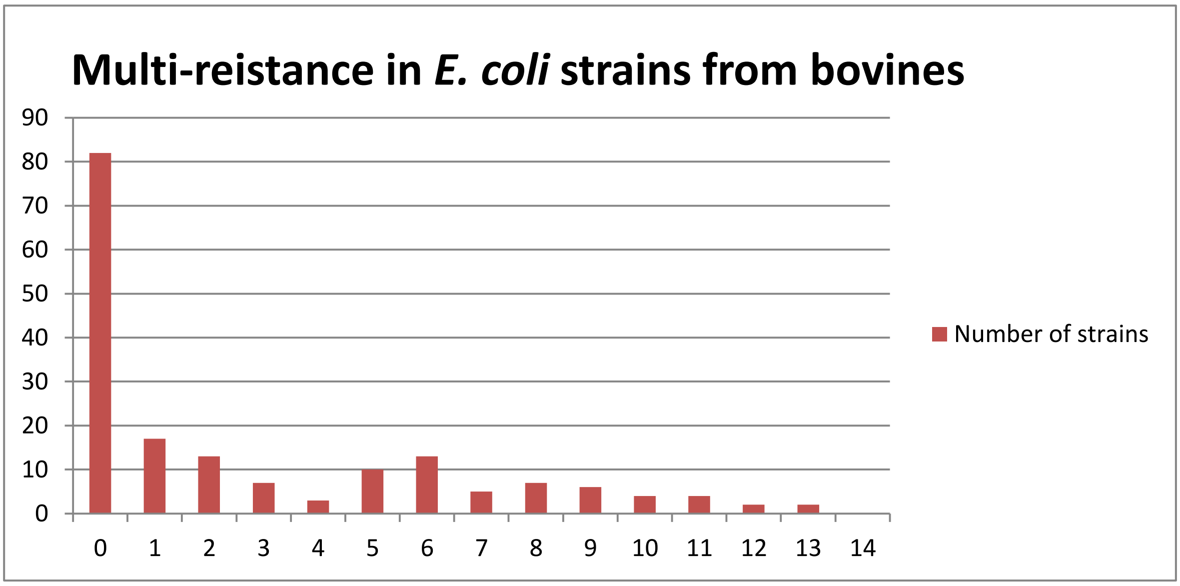

Figure 6. Cumulative percentage of multi-resistances from bovines

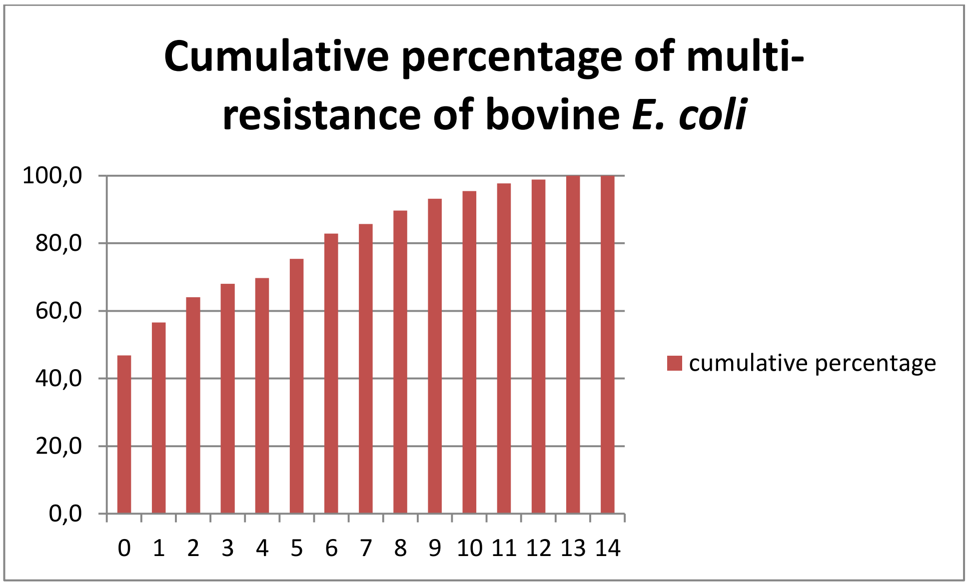

Table 9. Antibiotic resistance in commensal *E. coli* from veal calves

concentration	AMP	CHL	CIP	COL	FFN	FOT	GEN	KAN	NAL	SMX	Str	TAZ	TET	TMP
<=0.008	0	0	2	0	0	0	0	0	0	0	0	0	0	0
0.015	0	0	74	0	0	0	0	0	0	0	0	0	0	0
0.03	0	0	23	0	0	0	0	0	0	0	0	0	0	0
0.06	0	0	4	0	0	148	0	0	0	0	0	0	0	0
0.12	0	0	15	0	0	11	0	0	0	0	0	0	0	0
0.25	0	0	34	0	0	4	0	0	0	0	0	157	0	0
0.5	0	0	7	0	0	2	98	0	0	0	0	4	0	49
1	1	0	3	0	0	5	70	0	0	0	0	6	6	5
2	16	2	1	170	2	4	1	0	0	0	0	3	27	1
4	29	29	0	6	48	2	2	126	101	0	13	4	4	1
8	1	59	7	5	77	5	1	2	7	3	35	4	1	1
16	0	14	11	0	39	0	6	2	4	11	16	2	1	1
32	0	18	0	0	7	0	1	0	4	21	10	1	2	5
64	134	26	0	0	0	0	2	1	16	10	25	0	30	118
128	0	33	0	0	8	0	0	3	49	1	34	0	110	0
256	0	0	0	0	0	0	0	47	0	0	48	0	0	0
512	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1024	0	0	0	0	0	0	0	0	0	2	0	0	0	0
2048	0	0	0	0	0	0	0	0	0	133	0	0	0	0
Total	181	181	181	181	181	181	181	181	181	181	181	181	181	181
NR	134	77	82	11	15	18	12	53	69	136	117	20	143	126
%R	74,0	42,5	45,3	6,1	8,3	9,9	6,6	29,3	38,1	75,1	64,6	11,0	79,0	69,6
CI	67-80	35,2-50	37,9-53	3,1-11	4,7-13	6-15	3,5-11	22,8-36	31-46	68,2-81	57,2-72	6,9-17	72,3-85	62,4-76

Table 10. Multi-resistance in commensal *E. coli* from veal calves

Number of antimicrobials	Number of strains	Cumulative number of strains	Cumulative %
0	21	21	11,602
1	10	31	17,127
2	12	43	23,757
3	7	50	27,624
4	12	62	34,254
5	15	77	42,541
6	24	101	55,801
7	25	126	69,613
8	20	146	80,663
9	13	159	87,845
10	8	167	92,265
11	10	177	97,79
12	3	180	99,448
13	1	181	100
14	0	181	100

Figure 7. Number of strains with multi-resistances veal calves

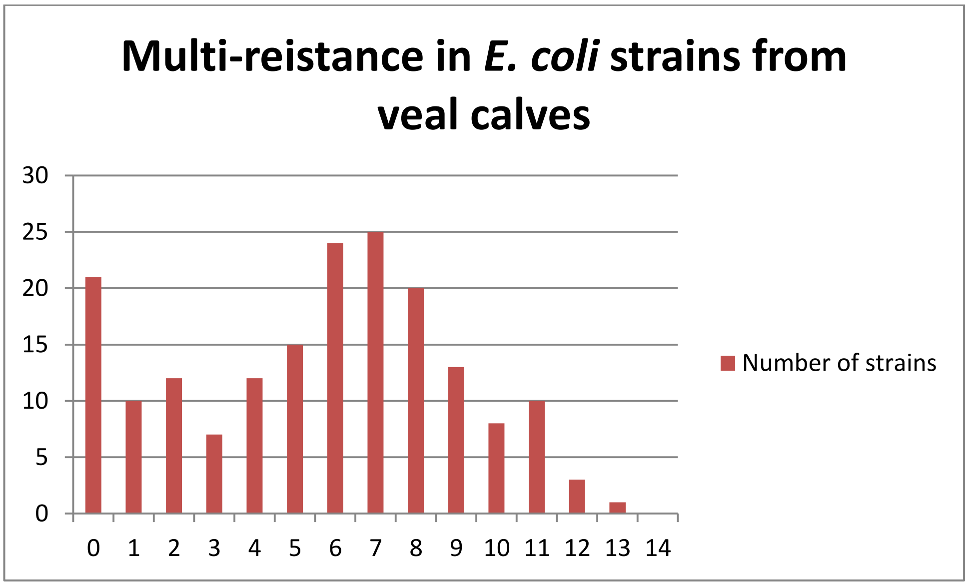

Figure 8. Cumulative percentage of multi-resistances veal calves

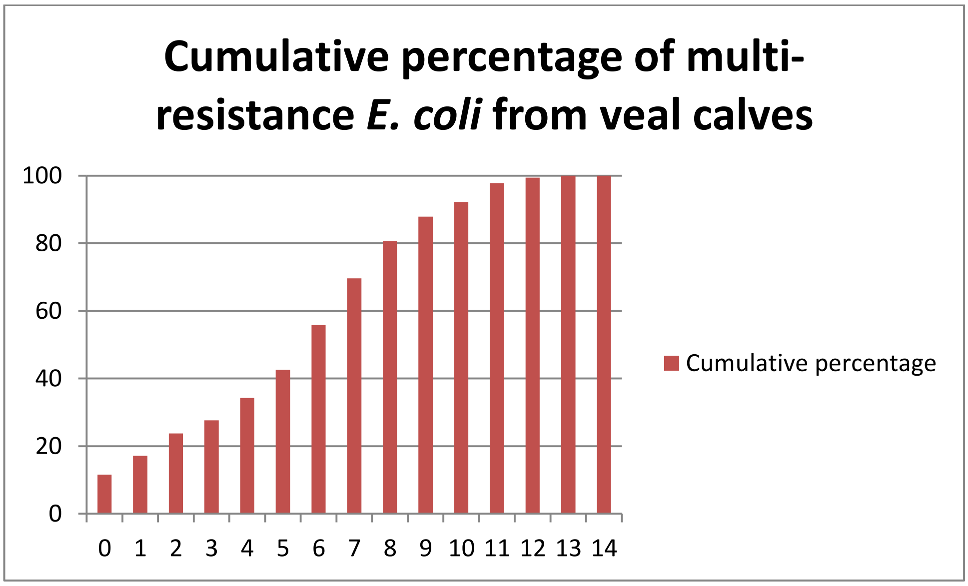

Table 9. Comparison 2011-2012, poultry.

	2011 (N=420)		2013 (N=324)		
	%R	CI	%R	CI	Chi square
AMP	85,0	81.2-88	80,6	75,8-85	NS
CHL	24,3	20.5-29	45,1	39,6-51	S
CIP	62,9	58-67	79,9	75,2-84	S
COL	0,5	0.1-2	4,6	2,6-8	S
FFN	0,7	0.1-2	3,7	1,9-6	NS
FOT	19,1	15.4-23	28,7	23,8-34	S
GEN	5,0	3.1-8	5,9	3,6-9	NS
KAN	6,9	4.7-10	14,2	10,6-18	S
NAL	63,1	58.3-68	78,1	73,2-82	S
SMX	74,3	69.8-78	80,6	75,8-85	NS
Str	60,1	55.1-65	82,1	77,5-86	S
TAZ	10,0	7.3-13	25,3	20,7-30	S
TET	64,8	60-69	69,4	64,1-74	NS
TMP	63,1	58.3-68	69,3	63,8-74	NS

Table 10. Comparison 2011-2012, pigs.

	2011 (N=157)		2012 (N=212)		
	%R	CI	%R	CI	Chi square
AMP	51,0	42.9-59	48,1	41,2-55	NS
CHL	26,8	20-34	29,2	23,2-36	NS
CIP	14,0	9-20	16,5	11,8-22	NS
COL	0,6	0-3	0,9	0,1-3	NS
FFN	4,5	1.8-9	4,7	2,3-9	NS
FOT	4,5	1.8-9	2,8	1-6	NS
GEN	4,5	1.8-9	0,9	0,1-3	NS
KAN	3,2	1-7	4,2	2-8	NS
NAL	12,7	8-19	11,8	7,8-17	NS
SMX	58,6	50.5-66	58,5	51,5-65	NS
Str	43,3	35.4-51	52,4	54,4-59	S
TAZ	2,5	0.7-6	3,8	1,6-7	NS
TET	56,7	48.6-65	59,4	52,6-66	NS
TMP	50,3	42.2-58	53,3	55,8-69	NS

Table 10. Comparison 2011-2012, bovines.

	2011 (N=154)		2012 (N=175)		
	%R	CI	%R	CI	Chi square
AMP	26,6	19.8-34	35,4	28,4-43	NS
CHL	14,3	9.2-21	17,7	12,4-24	NS
CIP	11,0	6.6-17	20,6	18,4-27	S
COL	0,6	0-4	2,9	0,9-7	NS
FFN	6,5	3.2-12	7,4	4-12	NS
FOT	4,5	1.8-9	6,3	3,2-11	NS
GEN	3,9	1.4-8	4,0	1,6-8	NS
KAN	5,2	2.3-10	13,1	8,5-19	NS
NAL	12,3	7.6-19	17,1	11,9-24	NS
SMX	28,6	21.6-36	42,3	34,9-52	NS
Str	23,4	16.9-31	37,1	30-45	NS
TAZ	3,9	1.4-8	7,4	4-12	NS
TET	19,5	13.5-27	36,0	28,9-44	S
TMP	19,5	13.5-27	28,6	22-36	NS

Table 10. Comparison 2011-2012, Veal calves.

	2011 (N=34)		2012 (N=181)		
	%R	CI	%R	CI	Chi square
AMP	70,6	52.5-85	74,0	67-80	NS
CHL	50,0	32.4-68	42,5	35,2-50	NS
CIP	41,2	24.6-59	45,3	37,9-53	NS
COL	14,7	5-31	6,1	3,1-11	NS
FFN	14,7	5-31	8,3	4,7-13	NS
FOT	0,0	0-10	9,9	6-15	NS
GEN	20,6	8.7-38	6,6	3,5-11	NS
KAN	29,4	15.1-47	29,3	22,8-36	NS
NAL	41,2	24.6-59	38,1	31-46	NS
SMX	79,4	62.1-91	75,1	68,2-81	NS
Str	52,9	35.1-70	64,6	57,2-72	NS
TAZ	0,0	0-10	11,0	6,9-17	NS
TET	73,5	55.6-87	79,0	72,3-85	NS
TMP	70,6	52.5-85	69,6	62,4-76	NS