



Report: antimicrobial resistance in commensal *E. coli* from poultry, pigs, cows and veal calves. 2013

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.

In this the report, the third 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 and was composed of faeces of at least 10 bovines.



2.1.4 Veal calves

Pooled fresh faecal material was collected at the abattoir from veal calves of less than 7 months of age. One sample originated from one farm.

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 McConkey 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) were merged to the administrative data 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 a 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).

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 Pearson's chi-square test, and where appropriate the Fischer exact test, significance of the differences were calculated.

Multi-resistance was determined by transforming the MIC data into resistant (R) and susceptible (S). Number of antimicrobials to which a strain was resistant were 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 234 strains were tested. Highest resistance was seen against ampicillin followed by ciprofloxacin, streptomycin, sulphonamide and nalidixic acid, closely followed by tetracycline and trimethoprim resistance. Nearly one third of the strains were resistant to chloramphenicol. Ten percent 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%. The combination of sulfnamide-trimethoprim resistance was calculated and was 59% (CI: 52.4-65).

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 (76.1%) is worrisome. It should be noted that this resistance is mainly low level resistance, mediated by one point mutation in the gyrase gene. High level resistance is mediated by two mutations (Rodriguez-Martinez et al., 2011). 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) (Rodriguez-Martinez et al., 2011). 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 (6) showed cross-resistance to chloramphenicol. It should be noted that chloramphenicol resistance remains high (32.5%). 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 (Schwarz et al., 2004). Florfenicol resistance is associated with multi-resistance (at least 7 antibiotics and up to 13, remaining only susceptible to colistin).

Resistance to the cephalosporins, cefotaxime and ceftazidime, is approximately 10%. 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, since it has been shown before that quite some strains carry an *ampC* gene. These cephalosporin resistant strains are clearly multi-resistant with all strains resistant to more than 5 antimicrobials.

Contrary to streptomycin (74.9%), there is little resistance to the other aminoglycosides tested (gentamicin and kanamycin). Colistin resistance remains negligible (2.1%). The strains are however associated with multi-resistance (2 strains are resistant 9 antibiotics, 2 to 10 antimicrobials and 1 to 11).

Resistance to sulphonamides and trimethoprim (about 70%) is also substantial. Resistance to the combination (as determined by resistance to both components) is evident in 60.2% of the strains (CI 53.7-66), which is also substantial.

Only 4.3% of the strains remained fully susceptible (Table 3). A little more than 50% of the strains was resistant to at least 6 different antimicrobials (figure 1). 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 13 antimicrobials tested, remaining susceptible to colistin only. Another strain was resistant to 12 antibiotics, remaining susceptible to colistin and gentamicin and one strain was resistant to 11 antibiotics remaining susceptible to cephalosporins and gentamicin.

3.2 Pigs

Susceptibility of commensal *E. coli* towards the different antimicrobials tested is shown in table 4. For three antimicrobials, resistance was higher than 50% (sulphonamides, streptomycin tetracycline). Trimethoprim and ampicillin resistance was just below 50%. The combination sulphonamide-trimethoprim was determined and 45.4% (CI 38.4-52) of the strains was resistant against the combination, which 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 five colistin resistant strains were variably multi-resistant with one strain being resistant to 10 antibiotics, one to 6 and two to 3 and one was only co-resistant to tetracyclines. Also cephalosporin resistance remains low. Resistance to chloramphenicol remains relatively high with a little more than a quarter of the strains being resistant. Also here, since florfenicol resistance is below 3%, 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 (Schwarz et al., 2004). Resistance against aminoglycosides, except for streptomycin, remains low. (gentamicin: 2.8% and kanamycin: 5.2%)

Ciprofloxacin (7.1%) resistance is higher than nalidixic acid resistance (3.8%). Also here the presence of plasmid mediated quinolone resistance (PMQR) is suspected in 7 strains.

Approximately a quarter of the strains remained fully susceptible. Half of the commensal *E. coli* from pigs were resistant to at least 3 antimicrobials. Two strains were resistant to as much as 11 antimicrobials (Table 6, figure 3 and 4). They had the same susceptibility profile and remained susceptible to colistin, and the cephalosporins.

3.3 Bovines

Results are shown in Table 9. Resistance in commensal *E. coli* from bovines is highest against sulphonamides (32.8%), followed by streptomycin (27.9%), tetracyclines (21.6%), trimethoprim (20.6%) and ampicillin (19.1%). Resistance against chloramphenicol was approximately 17% while florfenicol resistance was nearly 13%, which indicates that the contribution of the “old” chloramphenicol resistance gene in this population is proportionally smaller than in the strains of other animal populations (Schwarz et al, 2004). Other resistances are below 12% The combined resistance against trimethoprim-sulphonamides, as calculated from the combined resistance against the two components is 20.1% (CI:14.8-26), with only one strain resistant to trimethoprim only.

Seven strains were cefotaxime resistant of which 4 were ceftazidime susceptible. One ceftazidime resistant strain was cefotaxime susceptible. 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, with one strain resistant to ceftazidime only.

Colistin resistance (1.5%) was also associated with multi-resistance, ranging from 8 to 10 antibiotics.

More than half of the strains were fully susceptible (Table 10 and figure 7 and 8). However, some strains were highly multi-resistant, up to 13 antibiotics. The latter strain remained susceptible to colistin. The strain resistant to 12 antibiotics was additionally susceptible to ceftazidime. The diversity of resistance profiles of the resistant strains was large.

3.4 Veal calves

The highest prevalence of resistance in bovine commensal *E. coli* is against sulphonamides, tetracycline, trimethoprim, streptomycin and ampicillin with resistance percentages over 50%. Between one third and a quarter of of the strains is resistant against chloramphenicol, nalidixic acid, kanamycin and ciprofloxacin. Other resistances are less than 12.4% (Table 7). The combined resistance against trimethoprim-sulphonamides, as calculated from the combine resistance against the two components is 57.9% (CI: 50.8-



65). 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 3 strains also resistant to ceftazidime. Four ceftazidime resistant strains were not cefotaxime resistant. 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 the other strains with resistances to at least 6 different antibiotics, and the majority resistant against more than 9 antibiotics. One strain was resistant to only 3 antibiotics and this included florfenicol and ampicillin (which is cross resistant when cefotaxime resistant).

Also florfenicol resistance (12,4%) was associated with a high degree of multi-resistance. Similarly as in poultry and pigs, the "old" chloramphenicol resistance gene (Schwarz et al, 2004) is still largely present.

In 2013 12 colistin resistant strains were detected, and these were resistant to at least 7 antibiotics.

Also here, ciprofloxacin resistance (31.8%) was higher than nalidixic acid resistance (27.7%), indicating the presence of plasmid mediated quinolone resistance.

Less than a fifth of the strains are fully susceptible to all antimicrobials tested. Two strains are resistant to 13 different antimicrobials (Table 8 and figure 5 and 6). Those two strains were only susceptible to colistin. The one strain, resistant to 12 antimicrobials was susceptible to cefotaxime and gentamicin.

3.5 Comparison between animal species

Clearly, commensal *E. coli* from poultry are the most resistant strains with less than 5% of the strains remaining fully susceptible. Next in row are the strains from pigs (25.4%) and veal calves (17.3%). Strains from bovines are the most susceptible in this sample. A more half of the strains remain fully susceptible (59.3%), but multi-resistance can be up to 12 of the 14 tested antibiotics.

Resistance percentages to cephalosporins remain low in pigs, bovines and veal calves, while in poultry it is up to 10%. Chloramphenicol resistance is similar for the different origins (no significant difference). There is a large difference in ciprofloxacin resistance. While this type of resistance is very frequent in poultry (76.1%), it is significantly lower in veal calves (31.8%) and prevalence in pigs (6.8%) and bovines (10.8%) is lower than for veal calves. For pigs and bovines there is no significant difference. Colistin resistance remains low for *E. coli* from all animal species. Florfenicol resistance is typically associated with bovines (11.8%) and veal calves (12,4%) and is significantly higher than for poultry and pigs. Resistance to aminoglycosides with the exception of streptomycin, remains low for *E. coli* from all origins.

3.6 Conclusions



Resistance in commensal *E. coli* from different animal species remains problematic. Resistance against quite some antibiotics is so high that the efficacy of these antibiotics may be questioned. However, the extrapolation of the results on resistance in commensal *E. coli* to pathogenic strains (*E. coli* or other pathogenic bacteria) is difficult due to the scarcity of the data and the fact that different bacteria have different capabilities to take up resistance genes or have different rates of mutations leading to antimicrobial resistance. However, the resistance in commensal *E. coli* from veal calves is somehow lower compared to what has found in Belgium in pathogenic (neonatal) strains (Hendriksen et al., 2008). Since last year, samples are now only taken at the slaughterhouse, thus from the older animals, the age effect should be taken into account seen that in general, in the younger animals, resistance is higher.

Poultry strains remain the most resistant strains and show also the highest level of multi-resistance.

In bovines, resistance is lowest, however showing a large variety in multi-resistance with some strains highly multi-resistant.

Resistance against the older antibiotics remains high while against the more newly introduced antibiotics resistance remains quite low. An exception on this is colistin for which resistance remains low. At the other hand, chloramphenicol resistance remains at remarkably high levels seen it is not used anymore for over 20 years. Clearly, this is due to co-resistance selection. It indicates the complexity of the evolution of antimicrobial resistance in bacteria.



Table 1. List of abbreviations

Abbreviation	
AMP	Ampicillin
CHL	Chloramphenicol
CIP	Ciprofloxacin
COL	Colistin
FFN	Florfenicol
FOT	Cefotaxime
GEN	Gentamicin
KAN	Kanamycin
NAL	Nalidixic 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	3	0	0	0	0	0	0	0	0	0	0	0
0.015	0	0	38	0	0	0	0	0	0	0	0	0	0	0
0.03	0	0	15	0	0	0	0	0	0	0	0	0	0	0
0.06	0	0	3	0	0	188	0	0	0	0	0	0	0	0
0.12	0	0	15	0	0	19	0	0	0	0	0	0	0	0
0.25	0	0	85	0	0	3	9	0	0	0	0	202	0	0
0.5	0	0	19	0	0	1	168	0	0	0	0	7	0	90
1	2	0	14	0	0	4	43	0	0	0	0	8	7	3
2	17	3	12	230	3	5	2	0	0	0	0	7	79	0
4	15	45	6	3	74	2	1	203	57	0	14	0	8	1
8	2	105	14	1	121	12	3	10	10	12	33	5	0	0
16	1	5	10	0	31	0	3	3	3	22	14	5	0	0
32	1	14	0	0	3	0	3	1	3	27	12	0	1	1
64	196	24	0	0	1	0	2	3	46	11	28	0	35	139
128	0	38	0	0	1	0	0	1	115	1	27	0	104	0
256	0	0	0	0	0	0	0	13	0	0	106	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	0	0	0	0	0
>1024	0	0	0	0	0	0	0	0	0	160	0	0	0	0
Total	234	234	234	234	234	234	234	234	234	234	234	234	234	234
NR	198	76	178	4	5	24	12	21	164	162	173	25	140	141
%R	84,6	32,5	76,1	1,7	2,1	10,3	5,1	9,0	70,1	69,2	73,9	10,7	59,8	60,3
CI	79,4-88,7	26,8-38,8	70,1-81,1	0,6-4,5	0,9- 5,1	7,0-14,4	2,9-8,9	5,9-13,4	63,9-75,6	63-74,9	67,9-79,2	7,3-15,3	53,4-66	53,8-66,4



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

N antibiotics	N strains	% strains	Cumulative %
0	10	4,3	4,3
1	6	2,6	6,8
2	16	6,8	13,7
3	17	7,3	20,9
4	23	9,8	30,8
5	28	12,0	42,7
6	28	12,0	54,7
7	51	21,8	76,5
8	29	12,4	88,9
9	20	8,5	97,4
10	4	1,7	99,1
11	0	0,0	99,1
12	1	0,4	99,6
13	1	0,4	100,0
14	0	0,0	100,0



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

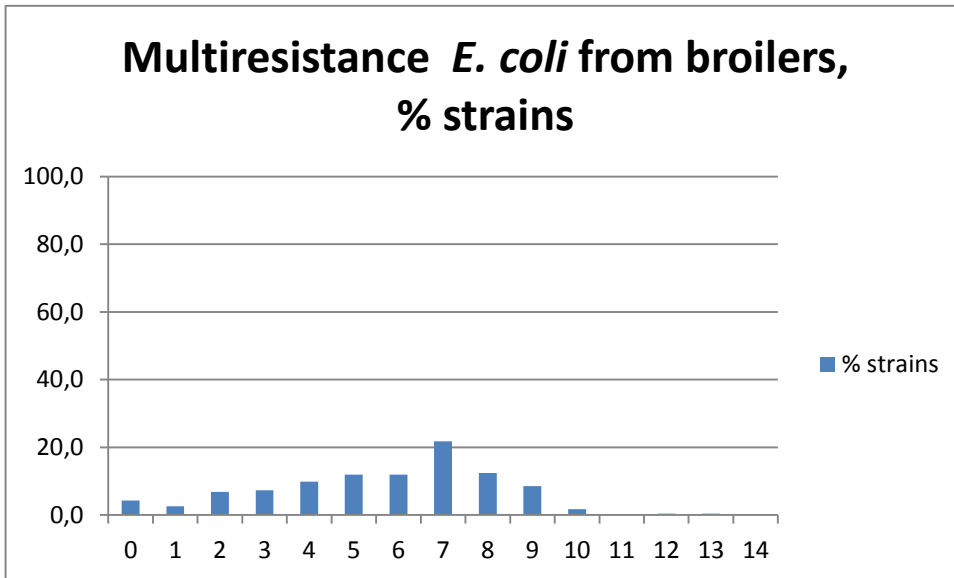


Figure 2. Cumulative percentage of multi-resistances from poultry

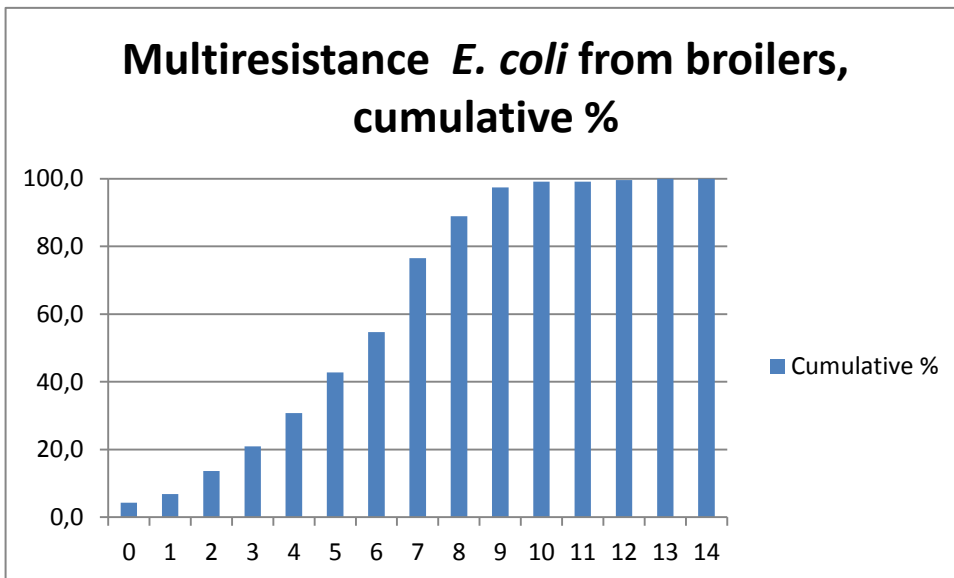




Table 4. Antimicrobial resistance in commensal *E. coli* from pigs

Concentration	AMP	CHL	CIP	COL	FFN	FOT	GEN	KAN	NAL	SMX	Str	TAZ	TET	TMP
<=0.008	0	0	13	0	0	0	0	0	0	0	0	0	0	0
0.015	0	0	140	0	0	0	0	0	0	0	0	0	0	0
0.03	0	0	39	0	0	0	0	0	0	0	0	0	0	0
0.06	0	0	2	0	0	193	0	0	0	0	0	0	0	0
0.12	0	0	4	0	0	9	0	0	0	0	0	0	0	0
0.25	0	0	6	0	0	2	9	0	0	0	0	200	0	0
0.5	2	0	0	0	0	0	126	0	0	0	0	3	0	101
1	2	0	0	0	0	0	61	0	0	0	0	1	17	5
2	50	3	1	201	8	1	6	0	0	0	0	0	72	0
4	59	32	0	4	60	1	1	190	195	0	8	1	6	1
8	0	110	1	1	105	0	0	5	2	16	64	1	3	1
16	1	7	0	0	29	0	0	4	1	35	18	0	0	0
32	0	11	0	0	2	0	1	1	0	26	25	0	3	1
64	92	24	0	0	0	0	2	1	3	17	40	0	30	97
128	0	19	0	0	2	0	0	0	5	2	24	0	75	0
256	0	0	0	0	0	0	0	5	0	1	27	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	0	0	0	0	0
>1024	0	0	0	0	0	0	0	0	0	108	0	0	0	0
Total	206	206	206	206	206	206	206	206	206	206	206	206	206	206
NR	93	54	14	5	4	2	4	11	8	112	116	3	108	100
%R	45,1	26,2	6,8	2,4	1,9	1,0	1,9	5,3	3,9	54,4	56,3	1,5	52,4	48,5
CI	38,4-52,1	20,6-32,7	4,1-11,2	1,0-5,7	0,7-5,1	0,2-3,8	0,7-5,1	3-9,4	1,9-7,6	47,5-61,1	49,4-63,0	0,5-4,5	45,6-59,2	41,7-55,4



Table 5. Multi-resistance in commensal *E. coli* from pigs

N antibiotics	N strains	% strains	Cumulative %
0	52	25,4	25,4
1	19	9,3	34,6
2	18	8,8	43,4
3	20	9,8	53,2
4	22	10,7	63,9
5	36	17,6	81,5
6	27	13,2	94,6
7	4	2,0	96,6
8	5	2,4	99,0
9	0	0,0	99,0
10	1	0,5	99,5
11	1	0,5	100,0
12	0	0,0	100,0
13	0	0,0	100,0
14	0	0,0	100,0



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

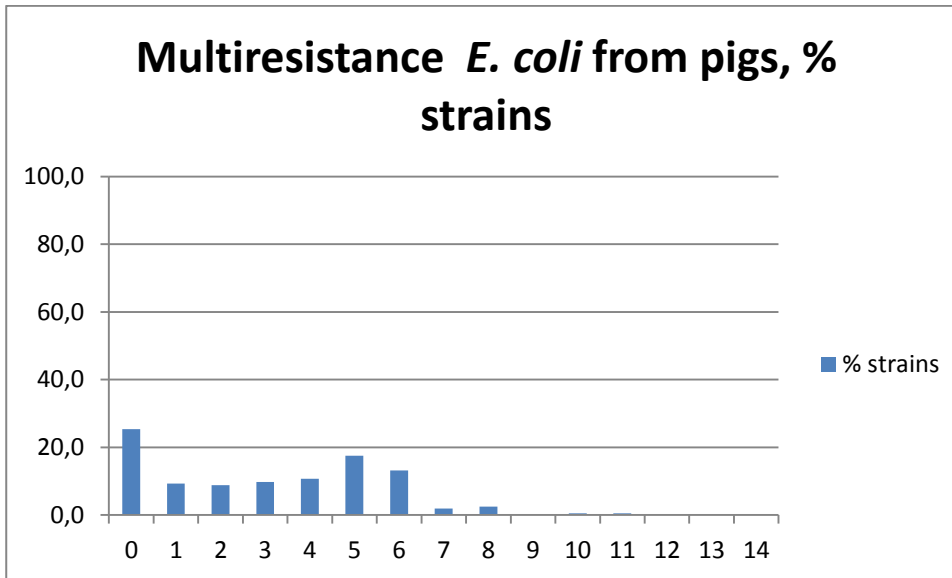


Figure 4. Cumulative percentage of multi-resistances from pigs

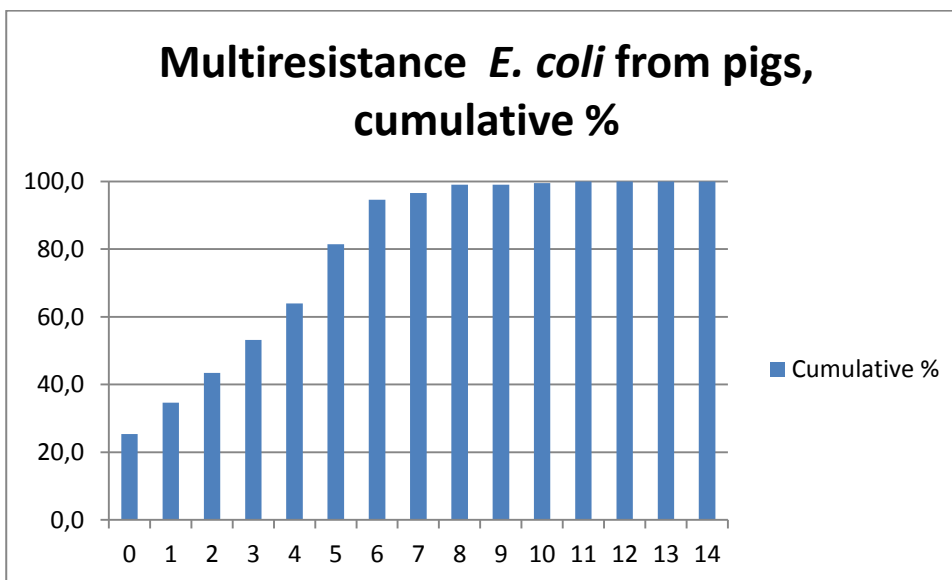




Table 6. Antibiotic resistance in commensal *E. coli* from bovines

Concentration	AMP	CHL	CIP	COL	FFN	FOT	GEN	KAN	NAL	SMX	Str	TAZ	TET	TMP
<=0.008	0	0	5	0	0	0	0	0	0	0	0	0	0	0
0.015	0	0	142	0	0	0	0	0	0	0	0	0	0	0
0.03	0	0	35	0	0	0	0	0	0	0	0	0	0	0
0.06	0	0	4	0	0	175	0	0	0	0	0	0	0	0
0.12	0	0	2	0	0	14	0	0	0	0	0	0	0	0
0.25	0	0	8	0	0	8	4	0	0	0	0	196	0	0
0.5	0	0	0	0	0	2	148	0	0	0	0	3	0	155
1	3	0	2	0	0	1	38	0	0	0	0	2	27	6
2	40	6	1	201	4	1	0	0	0	0	0	2	128	1
4	119	47	1	2	73	0	1	176	182	0	38	1	2	0
8	3	115	2	1	100	3	4	4	2	14	101	0	3	0
16	0	2	2	0	3	0	4	2	2	31	8	0	4	0
32	1	3	0	0	0	0	5	4	1	59	10	0	2	0
64	38	7	0	0	9	0	0	5	3	33	20	0	10	42
128	0	24	0	0	15	0	0	0	14	8	10	0	28	0
256	0	0	0	0	0	0	0	13	0	1	17	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
>1024	0	0	0	0	0	0	0	0	0	56	0	0	0	0
Total	204	204	204	204	204	204	204	204	204	204	204	204	204	204
NR	39	34	22	3	24	7	14	24	18	67	57	5	44	42
%R	19,1	16,7	10,8	1,5	11,8	3,4	6,9	11,8	8,8	32,8	27,9	2,5	21,6	20,6
CI	14,3-25,2	12,1-22,5	7,2-15,9	0,5-4,5	8,0-17,0	1,6-7,1	4,1-11,3	8,0-17,0	5,6-13,6	26,7-39,6	22,2-34,6	1,02-5,8	16,4-27,8	15,6-26,7



Table 7. Multi-resistance in commensal *E. coli* from bovines

N antibiotics	N strains	% strains	Cumulative %
0	121	59,3	59,3
1	23	11,3	70,6
2	5	2,5	73,0
3	8	3,9	77,0
4	4	2,0	78,9
5	9	4,4	83,3
6	7	3,4	86,8
7	7	3,4	90,2
8	7	3,4	93,6
9	3	1,5	95,1
10	5	2,5	97,5
11	3	1,5	99,0
12	1	0,5	99,5
13	1	0,5	100,0
14	0	0,0	100,0



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

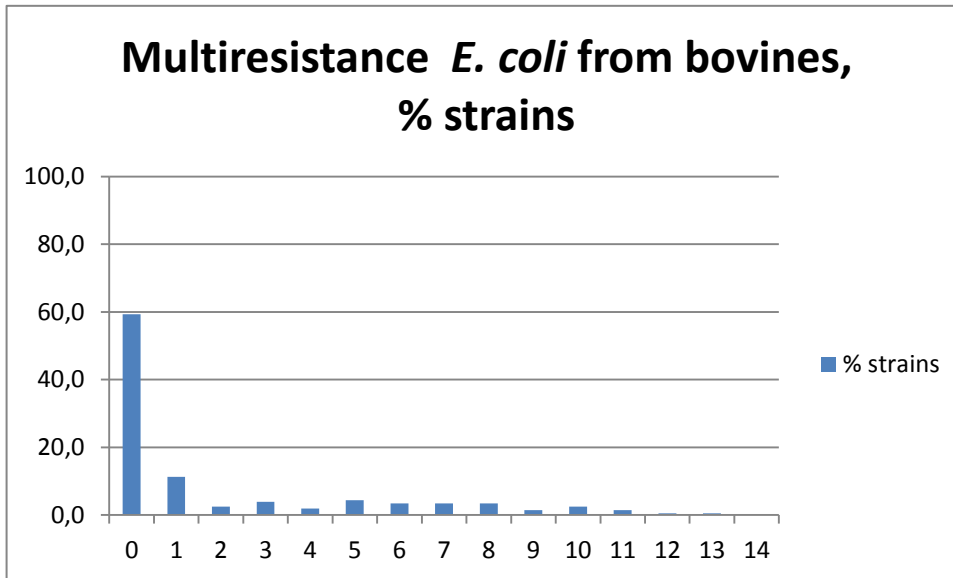


Figure 6. Cumulative percentage of multi-resistances from bovines

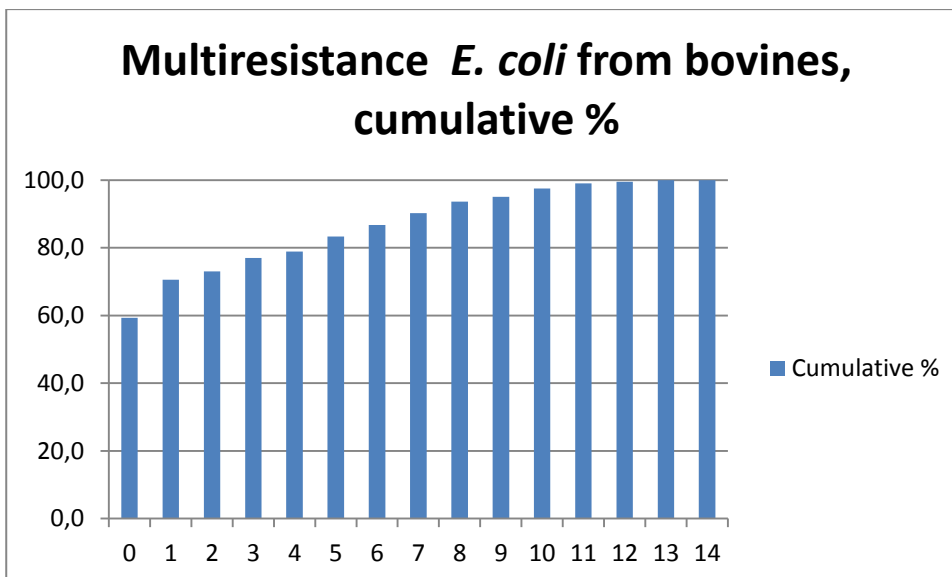




Table 8. Antibiotic resistance in commensal *E. coli* from veal calves

Concentration	AMP	CHL	CIP	COL	FFN	FOT	GEN	KAN	NAL	SMX	Str	TAZ	TET	TMP
<=0.008	0	0	5	0	0	0	0	0	0	0	0	0	0	0
0.015	0	0	101	0	0	0	0	0	0	0	0	0	0	0
0.03	0	0	31	0	0	0	0	0	0	0	0	0	0	0
0.06	0	0	9	0	0	168	0	0	0	0	0	0	0	0
0.12	0	0	8	0	0	19	0	0	0	0	0	0	0	0
0.25	0	0	15	0	0	8	13	0	0	0	0	182	0	0
0.5	0	0	4	0	0	3	128	0	0	0	0	12	0	79
1	2	0	2	0	0	2	42	0	0	0	0	2	11	4
2	22	1	2	190	0	0	3	0	0	0	0	3	34	2
4	44	34	0	8	63	0	1	146	138	0	14	3	2	2
8	4	86	8	4	82	2	1	6	2	4	51	0	0	0
16	1	13	16	0	32	0	3	1	6	20	23	0	0	0
32	1	8	0	0	9	0	4	0	5	26	13	0	4	3
64	128	11	0	0	2	0	7	0	6	10	19	0	31	112
128	0	49	0	0	14	0	0	0	45	2	29	0	120	0
256	0	0	0	0	0	0	0	49	0	0	53	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	0	0	0	0	0
>1024	0	0	0	0	0	0	0	0	0	140	0	0	0	0
Total	202	202	201	202	202	202	202	202	202	202	202	202	202	202
NR	130	68	64	12	25	7	16	50	56	142	114	8	155	117
%R	64,4	33,7	31,8	5,9	12,4	3,5	7,9	24,8	27,7	70,3	56,4	4,0	76,7	57,9
CI	57,4-70,7	27,4-40,5	25,7-38,7	3,4-10,2	8,5-17,7	1,7-7,1	4,9-12,6	19,3-31,2	21,9-34,4	63,6-76,2	49,5-63,2	2-7,8	70,3-82,1	50,9-64,6



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

N antibiotics	N strains	% strains	Cumulative %
0	35	17,3	17,3
1	15	7,4	24,8
2	5	2,5	27,2
3	14	6,9	34,2
4	22	10,9	45,0
5	27	13,4	58,4
6	20	9,9	68,3
7	20	9,9	78,2
8	15	7,4	85,6
9	11	5,4	91,1
10	8	4,0	95,0
11	7	3,5	98,5
12	1	0,5	99,0
13	2	1,0	100,0
14	0	0,0	100,0



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

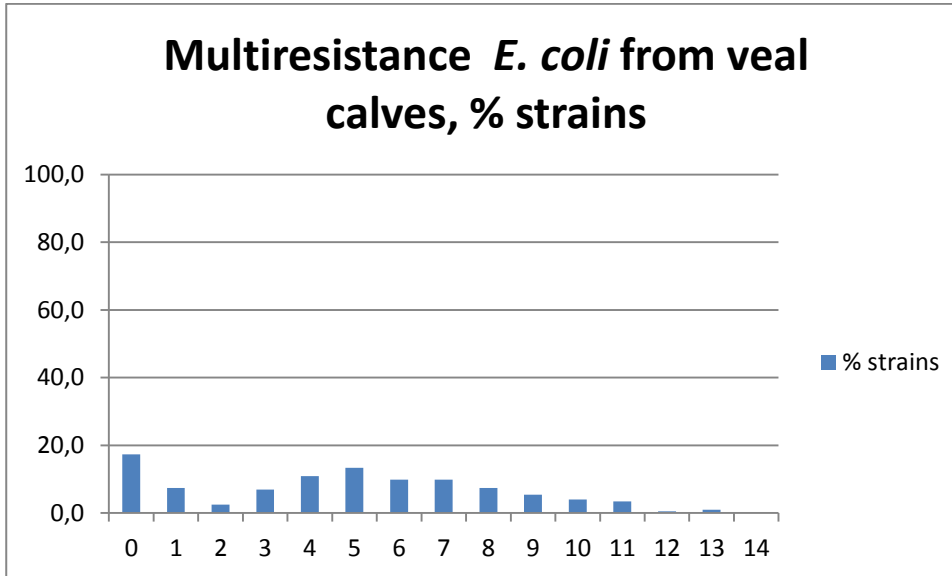
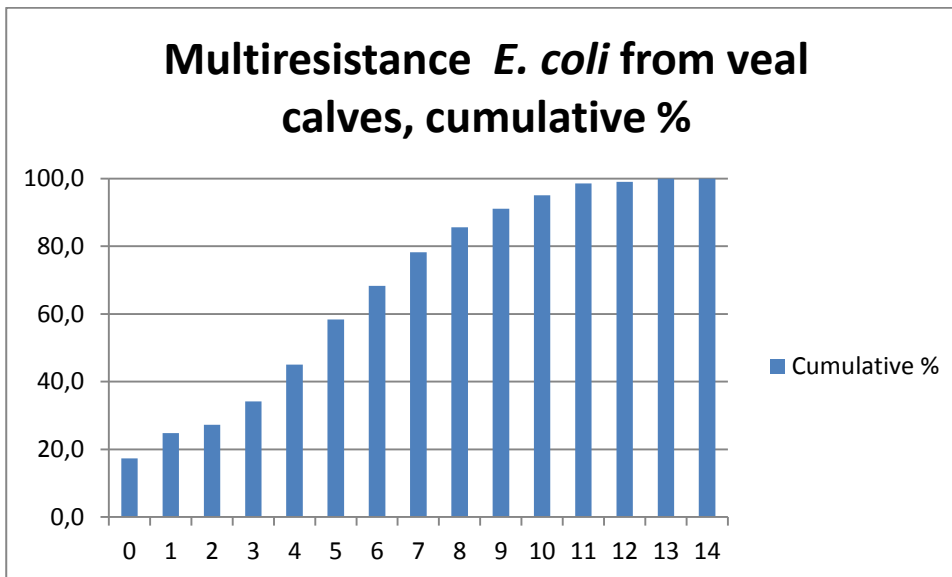


Figure 8. Cumulative percentage of multi-resistances veal calves





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