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Antimicrobial Resistance in commensal *Escherichia coli* from livestock in Belgium: *Trend Analysis 2011-2017*

EXECUTIVE SUMMARY

Background & objective
Belgian trend analysis of antimicrobial resistance in faecal *Escherichia coli* (*E. coli*) retrieved from livestock during seven consecutive years (2011-2017) was performed in accordance with the European legislation.

Methodology
Samples collected by the Federal Agency for the Safety of the Food Chain (FASFC) were taken at the slaughterhouse for veal calves (n=1160), broilers (n=1610) and fattening pigs (n=1300) and on farms for young beef cattle (n isolates=1173). Microbiology was performed according to standard procedures. *S*usceptibility was tested over consecutive years for 11±3 antimicrobial agents by a micro-dilution technique (Trek Diagnostics) and conversion of minimal inhibitory concentrations to binary qualitative values (Resistant/Susceptible) was done by means of the Epidemiological cut-offs values (ECOFFs) as defined by the European Committee on Antimicrobial Susceptibility Testing (EUCAST). Statistics were carried out using SAS 9.3 software and R freeware.

For each animal category and year, the proportion of resistant isolates (p) was calculated for the individual antimicrobial agents and 95% confidence interval (CI) were constructed for *logit(p)* to avoid interval boundaries outside the range [0-1]. Several statistical methods were used to model the probability of an isolate to be resistant: logistic regression models (in the univariate model each antimicrobial was considered separately), a linear Generalized Estimating Equations model (GEE) and non-linear mixed models (both multivariate models; taking into account the possible correlation between antimicrobial substances in a single model).
Similarly, multi-resistance (resistance to at least three antimicrobial families) was calculated and logistic regression models identified significant trends. Finally, a diversity index (weighted entropy) was calculated to describe the degree of diversity of multi-resistance.

Results
In **veal calves**, despite high levels of resistance (>50%) that were observed for the seven consecutive years for TET, SMX, AMP and a rise of 13.8% between 2016 and 2017 for TMP, the linear multivariate model (GEE) showed a statistically significant decrease of resistance over time for all tested substances but GEN, FOT and TAZ. Based on the non-linear mixed multivariate model a constant significant decrease in resistance (OR<1) for all substances from 2011 to 2014 is noticed. However, this significant decrease stopped from 2015 onwards for AMP, FOT, GEN, TAZ, and from 2016 onwards for CHL, CIP, COL, SMX, TET, and TMP. We should pay attention to these substances for which prevalence increased in 2017.
Globally, significantly lower prevalences of resistance were observed in *E. coli* from young **beef cattle** compared to veal calves, yet the same substances were involved: AMP, SMX, TET and TMP. Between 2016 and 2017, prevalence increased for CIP (+6.82%), FOT (+5.53%) and TMP (+5.57%). Based on the results of the linear multivariate model (GEE), the probability to be resistant significantly decreases over time for all tested substances except for CHL, FOT, TAZ and GEN. Based on the non-linear mixed multivariate model there is a constant significant

decrease resistance (OR<1) for AMP, CIP, NAL, TAZ from 2011 to 2014 and to 2015 for TMP and this decrease is followed by a continuous non-significant increase in resistance (OR>1 in 2017).

A high prevalence of resistance was observed for **broiler chickens** with values ≥ 50% for the seven consecutive years for AMP, CIP and SMX. Prevalence of resistance was observed with values ≥ 50% for six years for NAL and TMP. Prevalence of resistance increased from 2016 to 2017 by 9.95% and 8.63% for FOT and TAZ respectively. Globally, whatever the NL model used, there is a decreasing trend in resistance in CHL, CIP (significant), COL (significant), NAL (significant).

For **fattening pigs**, the prevalence of resistance for TET and SMX was above 40% during the seven consecutive years. AMP is in 2017 and for the first time, the antimicrobial with the highest prevalence. This increase is considered as significant by both NL models. A significant increase is also noted for AMP, CIP, FOT and TAZ by both NL models.
Based on the results of the linear multivariate model (GEE), a significant decrease of resistance over time was observed for SMX, TMP, TET, CIP and NAL.

The proportion of **multi-resistant strains** (= strains resistant to at least three antimicrobials) was very high for broiler chickens (>62%) and high for veal calves (>50%) during the seven consecutive years. After four consecutive years of decrease, multi-resistance increased in beef cattle in 2017 (+ 6.59%).
25.95%, 72.50%, 11.32%, 27.12%, of, respectively, calves, cattle, chicken and pig isolates, were fully susceptible (=no resistance) in 2017 to all tested antimicrobials.
From the linear and non-linear models and for all species, significant decreases in multi-resistance were observed from 2011 onwards but progressively faded out across the last few years.

1. CONTEXT

This report summarises the results of the trend analysis of the data related to antimicrobial resistance in *Escherichia coli* (*E. coli*) during seven consecutive years (2011-2017) regarding commensal intestinal flora of several livestock categories in Belgium:

- Veal calves
- Young beef cattle
- Fattening pigs
- Broiler chickens

Commensal *E. coli* is regarded as a general indicator for resistance amongst Gram-negative bacteria. It can be frequently isolated from all animal species is receptive for resistance determinants and is therefore suitable for comparisons and surveillance programmes. Earlier studies have shown that the aforementioned livestock categories undergo a substantial antimicrobial selection pressure in Belgium (Filippitzi M. E. et al., 2017).

During sampling, faecal material was taken at the slaughterhouse or directly at the farms depending on the animal category. *E. coli* isolated and thereafter tested for its susceptibility to a panel of several antimicrobial substances.

The objectives of this study were two-fold:

- To provide a **trend analysis of the prevalence of resistant strains** over the seven consecutive years, the results were compared and then analysed to check whether the observed trends (increase or decrease) were statistically significant.

- To evaluate the level of **multi-resistance and its trend** over the same period: using the same data, a calculation for each animal category for the proportion of multi-resistant strains was made (*i.e.* resistance to more than two antimicrobials (= at least three) by the same strain) and checked whether there was a significant trend.

2. MATERIAL AND METHODS

- **A. Sampling**

Samples of fresh faeces were collected each year by agents of the Federal Agency for the Safety of the Food Chain (FASFC) according to standardized technical sampling instructions (PRI codes) as part of a nationwide surveillance programme.

Samples were taken from the following categories of food-producing animals:

- **Veal calves**: calves kept in specialized units for fattening and slaughtered at an average age of 8 months. In 2011, faecal samples were taken on the floor at the farm level (PRI-516: 10 animals/farm of 7 months or younger), while the samples were taken directly from the rectum of the animals at the slaughterhouse (PRI-036: 1 animal sampled/farm)
- **Beef Cattle** (meat production): young animals (7 months or younger) from farms raising beef cattle for meat production. Faecal samples were taken from the floor at the farm (PRI-515: 1 sample consisted of a pool of faeces collected from different spots on the floor representing at least 10 animals).
- **Broiler chickens**: samples were taken at the slaughter house (PRI-019: pools of pairs of caeca from 10 chickens /batch)
- **Fattening pigs**: faecal samples of fattening pigs older than 3 months were taken from the rectum at the slaughterhouse (PRI-035: 1 animal /origin farm).

Following EFSA recommendations and in order to allow resistance trends to be detected with an acceptable confidence and precision (European Food Safety Authority (EFSA), 2008b), the target sample size for each animal category was fixed to 170 isolates.

In order to improve representativeness, the sampling was stratified by province proportionally to the number of registered herds or slaughterhouses.

- **B. Isolation of the strains and antimicrobial susceptibility testing**

Isolates of *E. coli* strains were obtained from the faecal samples. The isolations were performed by ARSIA except for PRI019, as of August 2017, analyses were performed at the laboratories of the Federal Agency for the Safety of the Food Chain at Melle and Gembloux, according to the standard operating procedures (SOP). The isolates were sent to the National Reference Laboratory (Sciensano) for bacterial species confirmation and antimicrobial susceptibility testing*. S*usceptibility was tested by a micro-dilution technique (Trek Diagnostics) as it is described in the annual reports. The antimicrobials common to the seven years (2011-2017) and those tested from 2014 to 2017 are presented in **Table A**. For each strain and each antimicrobial substance, the Minimal Inhibitory Concentration (MIC) was recorded: MIC is defined as the lowest concentration by which no visible growth could be detected. MICs were semi-automatically recorded and stored in a database (**Annexe 1)**.

Table A. Panel of antimicrobials tested during 2011-2017 for *E. coli*

Symbol	Antimicrobial
AMP	Ampicillin
AZI	Azithromycin
CHL	Chloramphenicol
CIP	Ciprofloxacin

COL	Colistin
FOT	Cefotaxime
GEN	Gentamicin
MER	Meropenem
NAL	Nalidixic acid
SMX	Sulphamethoxazole
TAZ	Ceftazidime
TET	Tetracycline
TIG	Tigecyclin
TMP	Trimethoprim

This table inventories all tested antimicrobial during the 7 consecutive years (in black) and from 2014 to 2017 (in green).

- **C. DATA**

The datasets for 2011-2017 were formatted in Excel files by the Department of Bacteriology of Sciensano and validated by the FASFC. They included identification of the samples corresponding to each isolate recorded in the interlaboratory software system (LIMS) merged with the corresponding MIC value for each tested antibiotic. After several steps of cross-checking and cleaning of the data, seven yearly data sets were produced, imported, validated and analysed in SAS 9.3 software and R freeware. Emphasis was put on verifying that the animal category of the sample was correct. The final annual datasets contained the following fields: i. isolate identification number, ii. animal category, iii. sampling date and iiii. MIC values for each of the tested antimicrobials (µg/mL).

- **Statistical Methods**

All subsequent statistics were carried out using SAS 9.3 software and R freeware.

A. Prevalence

Quantitative MIC values were converted into binary qualitative values (Resistant/Susceptible) based on the susceptibility breakpoints defined by the European Committee on Antimicrobial Susceptibility Testing (EUCAST)(European Committee on antimicrobial susceptibility testing). The ECOFFs (Epidemiological cut-offs values) were used in order to define strains as Resistant (R) or Susceptible (S) (**Annexe 1**).

For each animal category and year, the proportion of resistant isolates (p) was calculated per tested antimicrobial (resistance prevalence), as well as the associated 95% confidence interval (CI). In order to avoid interval boundaries outside 0-1, which does not make sense for probabilities, CI were constructed for *logit*(p).

B. Trend Analysis

The trends analysis aims at finding models to describe the variation of antimicrobial resistance over the years and to check if any change in resistance proportion is significant or not. For the antimicrobials common to the seven years, several statistical methods were used to model the probability of an isolate to be resistant: logistic regression models (in the univariate model each antimicrobial was considered separately), a linear Generalized Estimating Equations model (GEE) and a non-linear mixed model (both multivariate models; taking into account the possible correlation between antimicrobial substances in a single model; assuming an unstructured correlation matrix in the GEE).

The results are described in the form of Odds Ratio (OR), where an OR > 1 means that the probability to be resistant increases with time. Plots representing the log odds for each year were also produced for each antimicrobial and animal category. The odds represent here the probability to be resistant on the probability to be susceptible.

In this study, the effects of the different antimicrobials were assessed on an individual level. Hence, the 5% significance levels were specified for each antimicrobial separately. If the interest is in making a statement on the entire pool of antimicrobials jointly, a family wise significance level should be specified. In order to adjust the p-values and reduce the chances of obtaining false-positive results (type I errors; i.e. detection of a trend when in reality there is no trend) when several dependent or independent statistical tests are being performed simultaneously on a single data set, both the Bonferroni's correction method and the linear step-up method of Benjamini and Hochberg (1995) (Benjamini Y. and Hochberg Y., 1995) were applied to the GEE (linear multivariate model) and the resulting corrected p-values were produced and presented in annex for documentation.

C. Multi-resistance

Considering Multi-resistance was considered in this report as resistance by an isolate to at least three antimicrobials belonging to any three antimicrobial families as recommended by EFSA (European Food Safety Authority (EFSA), 2014, European Food Safety Authority (EFSA), 2008a). Considering the antimicrobials common to the seven years, these antimicrobials were: ampicillin, cefotaxime and/or ceftazidime, chloramphenicol, ciprofloxacin and/or nalidixic acid, colistine, gentamycin, sulphonamides, tetracycline and trimethoprim. In total 11 antibiotics belonging to 9 different classes were considered in this part of the analyses.
Based on this, for each animal category, the estimate for the prevalence of multi-resistant isolates was calculated together with the 95% CI, calculated using normal distribution.
In addition, logistic regression models were used to check whether there was a significant trend over the years regarding the prevalence of multi-resistant strains, for each animal category.
In addition, a diversity index was calculated for multi-resistance:

- Diversity index: Weighted entropy

This index is calculated to describe the degree of diversity of multi-resistance for a specific year and a specific animal category. The weighted entropy index takes into account order and will take higher values when multi-resistance is more frequent for large numbers of antimicrobials. Therefore, a higher weighted entropy index reflects a shift to multi-resistance to a greater number of antibiotics. This latter index was calculated using R software based on the formula of Guiasu (Guiaşu S., 1971).

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D. RESULTS

A. Prevalence

The following table (Table 1) summarizes the data obtained from 2011 to 2017 regarding prevalence of resistant isolates for each animal category and each tested antimicrobial substance.

Table 1. Prevalences of resistance by antimicrobial substance (%), by animal category and by year.

Category		2011				2012				2013				2014				2015				2016				2017			
		N	% resistance			N	% resistance			N	% resistance			N	% resistance			N	% resistance			N	% resistance			N	% resistance		
			Mean	L.C.I.	U.C.I.		Mean	L.C.I.	U.C.I.		Mean	L.C.I.	U.C.I.		Mean	L.C.I.	U.C.I.		Mean	L.C.I.	U.C.I.		Mean	L.C.I.	U.C.I.		Mean	L.C.I.	U.C.I.
Veal calves	AMP	34	70,59	52,45	83,93	181	74,03	67,09	79,95	202	64,36	57,46	70,71	188	54,79	47,56	61,82	196	57,65	50,57	64,44	174	55,75	48,22	63,02	185	58,38	51,08	65,33
	AZI		/	/	/		/	/	/		/	/	/		3,19	1,43	6,97		4,08	2,04	7,99		4,02	1,92	8,25		4,86	2,53	9,14
	CHL		50,00	33,00	67,00		42,54	35,48	49,92		33,66	27,44	40,52		25,53	19,76	32,31		27,55	21,70	34,28		25,29	19,33	32,34		28,65	22,54	35,64
	CIP		41,18	25,42	58,98		43,09	36,01	50,47		27,36	21,60	34,00		22,34	16,91	28,91		23,98	18,47	30,51		19,54	14,26	26,18		21,62	16,23	28,20
	COL		14,71	5,96	31,91		6,08	33,78	10,69		5,94	3,39	10,21		2,66	1,10	6,28		2,04	0,76	5,35		1,72	0,55	5,26		1,08	0,27	4,27
	FOT		0,00	/	/		9,94	6,33	15,29		3,47	1,65	7,13		0,53	0,07	3,74		3,06	1,37	6,69		4,02	1,92	8,25		4,32	2,16	8,46
	GEN		20,59	9,75	38,37		6,63	37,83	11,37		7,92	4,89	12,59		5,85	3,25	10,31		6,63	3,87	11,14		4,02	1,92	8,25		5,41	2,92	9,81
	MER														0,00	/	/		0,00	/	/		0,00	/	/		0,00	/	/
	NAL		41,18	25,42	58,98		38,12	31,28	45,47		27,72	21,94	34,35		20,74	15,50	27,20		21,94	16,65	28,33		18,39	13,27	24,92		11,35	7,49	16,84
	SMX		79,41	61,63	90,25		75,14	68,26	80,94		70,30	63,58	76,24		57,45	50,21	64,38		56,12	49,04	62,97		59,77	52,25	66,86		57,84	50,54	64,81
	TAZ		0,00	/	/		11,05	7,21	16,57		3,96	1,98	7,76		0,53	0,07	3,74		3,57	1,70	7,35		2,87	1,19	6,77		4,86	2,53	9,14
	TET		73,53	55,45	86,11		79,01	72,39	84,37		76,73	70,35	82,09		68,09	61,02	74,41		61,22	54,16	67,84		70,69	63,43	77,03		65,95	58,76	72,47
	TIG														0,00	/	/		0,00	/	/		0,00	/	/		0,00	/	/
	TMP		70,59	52,45	83,93		69,61	62,46	75,93		57,92	50,94	64,59		51,06	43,88	58,20		40,31	33,61	47,38		40,80	33,68	48,33		54,59	47,31	61,69
Beef cattle	AMP	154	25,32	19,02	32,87	175	35,43	28,64	42,86	204	19,12	14,25	25,15	164	20,73	15,15	27,69	180	14,44	9,99	20,44	176	15,34	10,70	21,52	120	20,00	13,70	28,24
	AZI		/	/	/		/	/	/		/	/	/		0,61	0,08	4,28		1,11	0,27	4,39		1,14	0,28	4,49		5,00	2,24	10,80
	CHL		14,29	9,55	20,83		17,71	12,70	24,16		16,67	12,12	22,48		15,85	10,98	22,34		10,56	6,81	16,02		10,23	6,51	15,71		15,00	9,60	22,68
	CIP		11,04	6,94	17,12		18,29	13,19	24,79		8,82	5,61	13,62		8,54	5,09	13,97		4,44	2,22	8,69		5,68	3,07	10,29		12,50	7,62	19,83
	COL		0,65	0,09	4,56		2,86	1,18	6,73		1,47	0,47	4,50		0,61	0,08	4,28		0,00	/	/		0,57	0,08	3,99		0,00	/	/
	FOT		4,55	2,16	9,30		6,29	3,49	11,05		3,43	1,63	7,06		2,44	0,91	6,38		3,33	1,49	7,27		1,14	0,28	4,49		6,67	3,34	12,88
	GEN		2,60	0,97	6,79		4,00	1,9	8,21		6,86	4,09	11,30		4,88	2,44	9,51		5,00	2,61	9,38		3,98	1,89	8,16		5,83	2,78	11,85
	MER		/	/	/		/	/	/		/	/	/		0,00	/	/		0,00	/	/		0,00	/	/		0,83	0,11	5,82
	NAL		11,69	7,45	17,87		17,14	12,21	23,53		8,82	5,61	13,62		7,32	4,18	12,51		3,89	1,85	7,98		5,11	2,66	9,59		9,17	5,11	15,92
	SMX		30,52	23,69	38,32		42,29	35,12	49,80		32,84	26,70	39,64		23,78	17,83	30,97		25,00	19,17	31,91		26,70	20,64	33,79		22,50	15,82	30,96
	TAZ		3,90	1,74	8,47		7,43	4,34	12,43		2,45	1,02	5,79		2,44	0,91	6,38		2,78	1,15	6,55		0,57	0,08	3,99		5,00	2,24	10,80
	TET		19,48	13,92	26,58		36,00	29,17	43,45		21,57	16,42	27,80		18,29	13,05	25,04		15,56	10,92	21,67		19,32	14,10	25,89		19,17	13,01	27,33
	TIG		/	/	/		/	/	/		/	/	/		0,00	/	/		0,00	/	/		0,00	/	/		0,83	0,11	5,82
	TMP		19,48	13,92	26,58		28,57	22,31	35,78		20,59	15,55	26,74		15,24	10,47	21,66		10,56	6,81	16,02		11,93	7,88	17,67		17,50	11,63	25,49
Chickens	AMP	420	84,76	80,98	87,90	320	81,56	76,90	85,46	234	84,62	79,36	88,72	158	72,78	65,24	79,21	152	75,66	68,12	81,89	167	83,83	77,37	88,72	159	76,73	69,45	82,71
	AZI		/	/	/		/	/	/		/	/	/		4,43	2,11	9,07		3,29	1,36	7,73		1,20	0,30	4,72		2,52	0,94	6,58
	CHL		24,29	19,41	28,64		45,94	40,52	51,45		32,48	26,75	38,79		20,89	15,19	28,01		19,08	13,54	26,20		25,15	19,10	32,36		24,53	18,41	31,89
	CIP		62,86	58,11	67,37		79,06	74,23	83,19		74,79	68,78	79,97		69,62	61,93	76,35		63,82	55,79	71,14		57,49	49,79	64,83		57,86	49,97	65,37
	COL		0,48	0,12	1,89		4,69	2,84	7,65		1,71	0,64	4,50		0,00	/	/		0,00	/	/		0,00	/	/		0,00	/	/
	FOT		19,05	15,56	23,11		29,38	24,62	34,63		10,26	6,95	14,89		8,86	5,29	14,48		4,61	2,19	9,42		10,18	6,39	15,83		20,13	14,55	27,16
	GEN		4,05	2,53	6,43		6,25	4,06	9,51		5,13	2,92	8,85		5,70	2,97	10,65		7,24	4,03	12,67		3,59	1,61	7,83		7,55	4,31	12,89
	MER		/	/	/		/	/	/		/	/	/		0,00	/	/		0,00	/	/		0,00	/	/		0,00	/	/
	NAL		62,86	58,11	67,37		78,44	73,57	82,62		70,09	63,86	75,64		63,29	55,42	70,51		61,84	53,79	69,29		48,50	40,94	56,14		52,83	44,98	60,54
	SMX		75,00	70,62	78,92		81,25	76,57	85,18		69,23	62,98	74,85		58,23	50,31	65,74		68,42	60,52	75,38		68,86	61,37	75,48		62,26	54,40	69,53
	TAZ		17,14	13,82	21,07		25,94	21,41	31,05		10,68	7,30	15,37		7,59	4,34	12,97		4,61	2,19	9,42		8,98	5,46	14,43		17,61	12,40	24,41
	TET		64,76	60,05	69,20		70,63	65,37	75,38		59,83	53,37	65,96		45,57	37,89	53,47		48,68	40,75	56,69		51,50	43,86	59,06		50,31	42,51	58,10
	TIG		/	/	/		/	/	/		/	/	/		0,00	/	/		0,00	/	/		0,00	/	/		3,14	0,28	4,46
	TMP		63,10	58,35	67,60		70,22	64,94	75,01		60,26	53,80	66,37		49,37	41,57	57,20		53,95	45,90	61,79		56,89	49,19	64,26		50,31	42,51	58,10
Pigs	AMP	157	49,04	41,23	56,91	217	47,47	40,85	57,17	206	45,15	38,43	52,05	184	41,30	34,35	48,62	186	35,48	28,88	42,69	173	46,82	39,44	54,35	177	51,98	44,56	59,31
	AZI		/	/	/		/	/	/		/	/	/		1,09	0,27	4,29		0,00	/	/		1,16	0,29	4,56		2,26	0,84	5,92
	CHL		26,75	20,35	34,30		28,57	22,91	34,99		26,21	20,62	32,70		28,80	22,67	35,83		17,74	12,86	23,97		24,28	18,41	31,29		25,99	20,01	33,02
	CIP		14,10	9,43	20,57		13,36	9,42	18,62		5,83	3,32	10,02		2,17	0,81	5,70		2,15	0,80	5,64		5,78	3,12	10,47		9,60	6,03	14,97
	COL		0,64	0,09	4,47		0,92	0,23	3,65		2,43	1,01	5,74		0,54	0,08	3,82		0,00	/	/		1,16	0,29	4,56		1,13	0,28	4,46
	FOT		4,46	2,12	9,12		2,76	1,24	6,05		0,97	0,24	3,84		0,54	0,08	3,82		1,08	0,27	4,25		2,31	0,86	6,05		10,73	6,92	16,28
	GEN		3,82	1,71	8,31		0,92	0,23	3,65		1,94	0,72	5,10		2,17	0,81	5,70		1,61	0,52	4,93		0,58	0,08	4,06		1,13	0,28	4,46
	MER		/	/	/		/	/	/		/	/	/		0,00	/	/		0,00	/	/		0,00	/	/		0,00	/	/
	NAL		11,46	7,31	17,54		12,90	9,03	18,10		3,88	1,94	7,61		1,09	0,27	4,29		1,61	0,52	4,93		1,73	0,55	5,29		4,52	2,26	8,83
	SMX		58,60	50,66	66,12		58,06	51,34	64,50		54,37	47,47	61,10		52,17	44,89	59,36		43,01	36,02	50,29		50,29	42,81	57,75		48,02	40,69	55,44
	TAZ		4,46	2,12	9,12		3,69	1,84	7,23		1,46	0,47	4,46		0,54	0,08	3,82		1,08	0,27	4,25		2,31	0,86	6,05		10,17	6,47	15,63
	TET		56,69	48,75	64,30		58,06	51,34	64,50		52,43	45,55	59,22		44,02	36,96	51,34		40,32	33,45	47,59		46,82	39,44	54,35		47,46	40,14	54,89
	TIG		/	/	/		/	/	/		/	/	/		0,00	/	/		0,00	/	/		0,00	/	/		1,13	0,28	4,46
	TMP		50,32	42,47	58,15		52,53	45,83	59,15		48,54	41,73	55,41		50,00	42,76	57,24		38,17	31,41	45,42		45,09	37,76	52,63		45,76	38,50	53,21

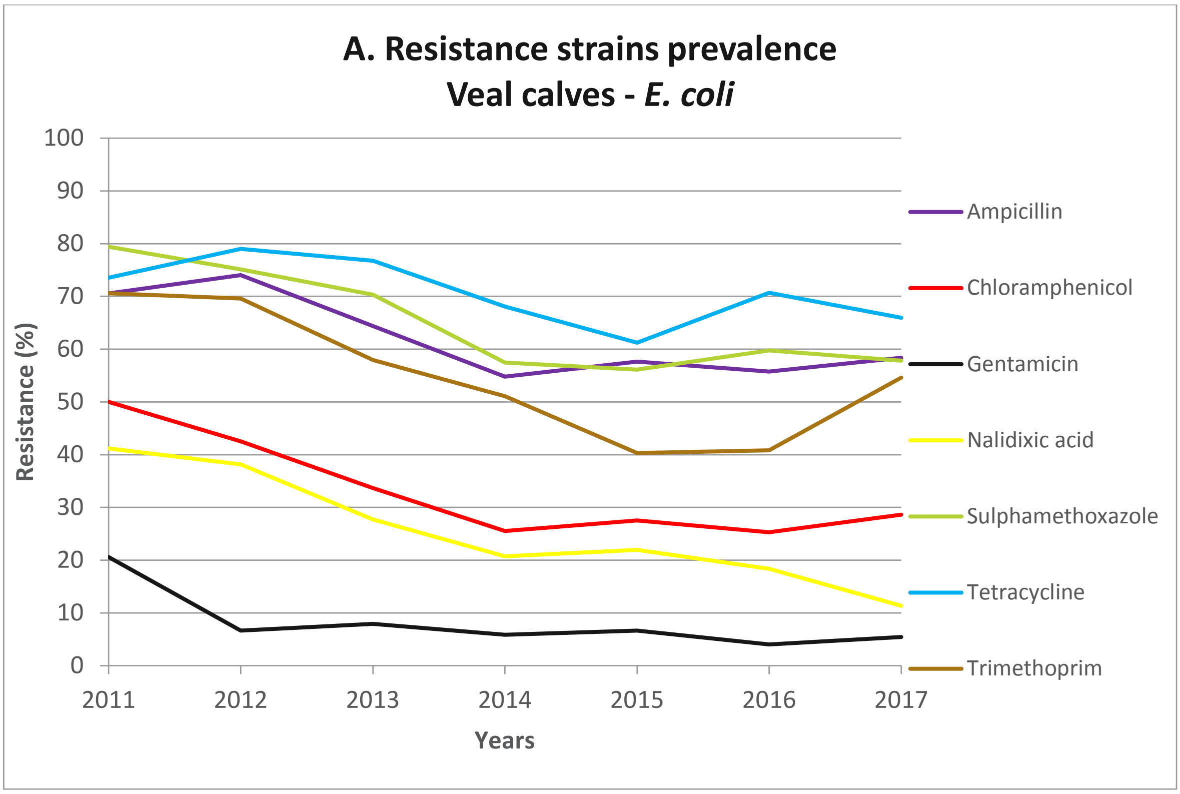
- Category: veal calves= veal calves = calves at slaughter aged < 8 months; beef cattle= young bovines for meat production < 7 month on farm; chickens= broiler chickens; pigs= fattening pigs at slaughter, older than 3 months.
- AMP: ampicillin; AZI: Azithromycin; CHL: chloramphenicol; CIP: ciprofloxacin; COL: colistin; FOT: cefotaxime; GEN: gentamicin; NAL: nalidixic acid; SMX: suphamethoxazole; TAZ: ceftazidime; TET: tetracycline; TMP: trimethoprim.
- N= number of tested samples.
- % resistance: mean prevalence of resistant isolates and confidence intervals (L.C.I.: lower confidence interval and U.C.I.: upper confidence interval) in per cent (%).

B.Trend analysis

Detailed outputs of the multiple comparisons corrections are presented in **Annex 2**. In this report the adjective 'high' was used in case of a prevalence of resistant strains higher than 50%. However, the significance of a given level of resistance will depend on the particular antimicrobial and its importance in human and veterinary medicine.

a) **Veal Calves**: (N= 34 (2011); 181 (2012); 202 (2013); 188 (2014); 196 (2015);174 (2016); 185 (2017)

As shown in **figures 1a**, in veal calves high levels of resistance (>50%) were observed for the seven consecutive years for TET, SMX, AMP. For TMP, resistance was > 40% for the seven consecutive years and >50% in 2011,2012,2013,2017 (+13.8% between 2016 and 2017). **Figure 1b**, shows the critical antimicrobials, Based on the World Health Organisation antimicrobials classification (World Health Organisation, 2017), **figure 1b** shows that resistance is globally decrease for NAL and CIP and remains low for the others.



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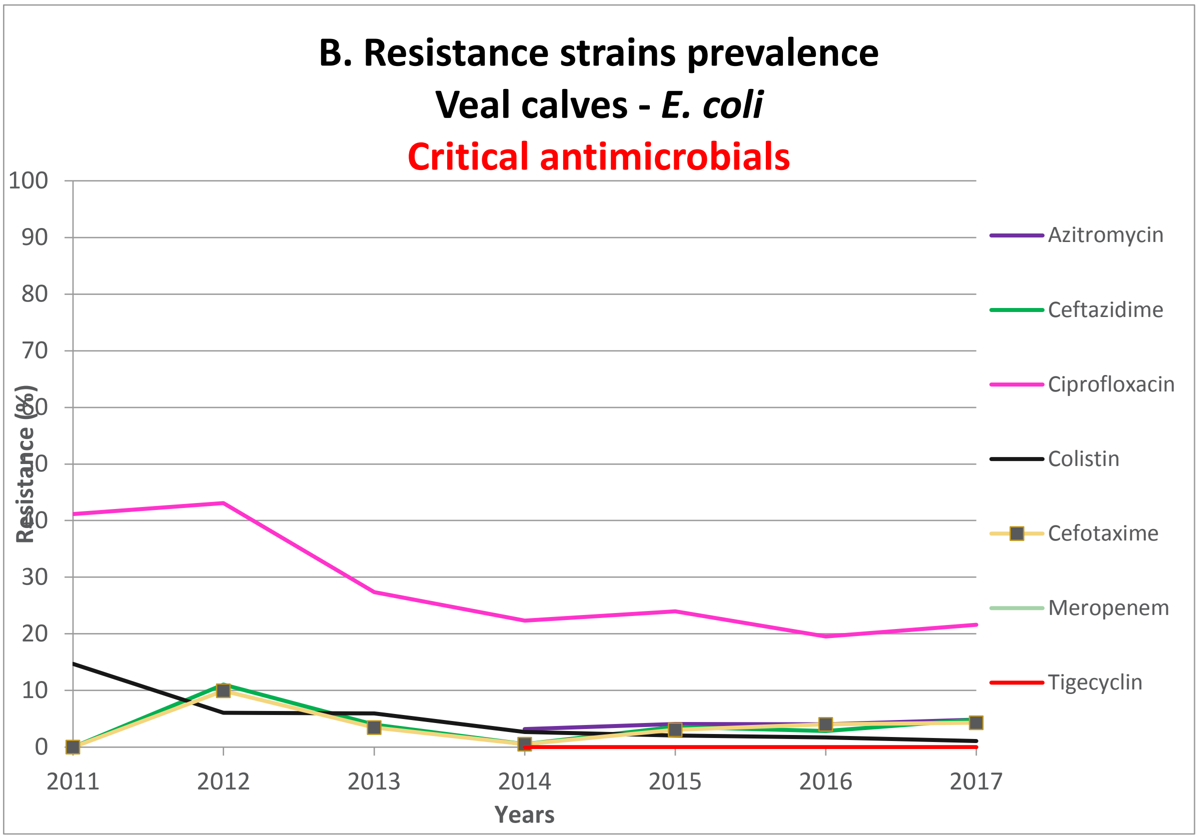


Figures 1a and 1b. Resistance strains prevalence: veal calves

Figures 1a and 1b describe the antimicrobial susceptibility trends of faecal *E. coli* retrieved from veal calves in Belgium (2011-2017).

Based on the results of the linear multivariate model (GEE), the probability to be resistant decreases significantly over time (2011-2017) for all tested substances except for GEN, FOT, TAZ (**figure 2**).

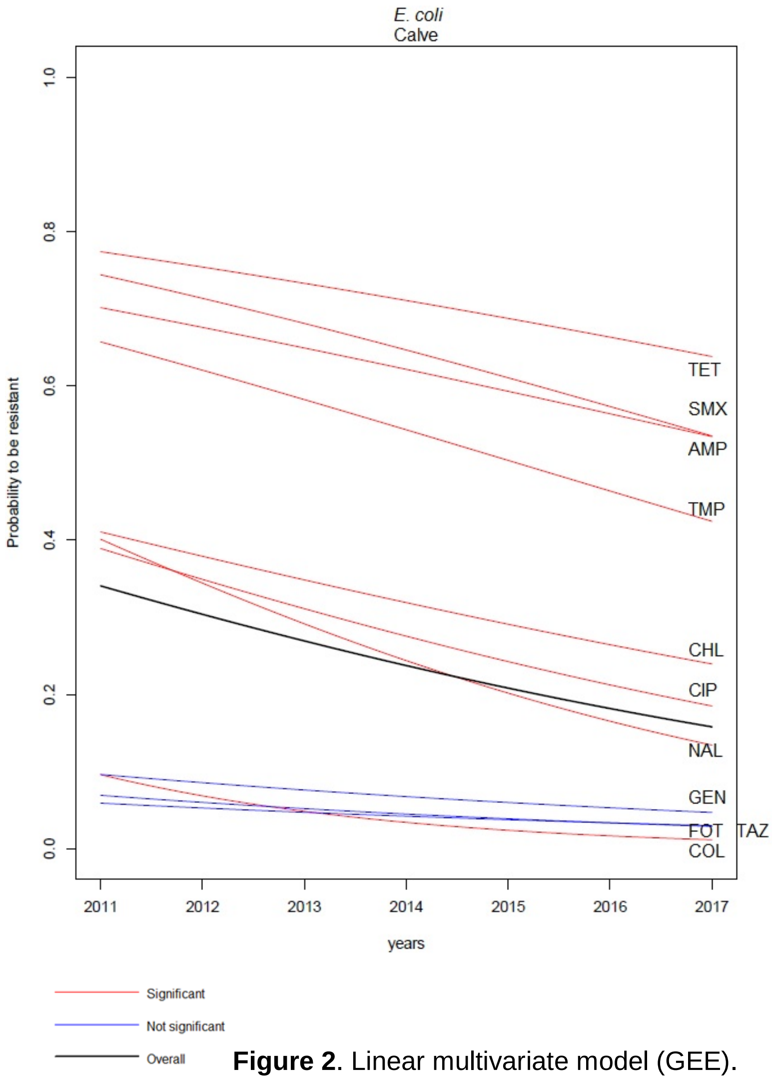


Figure 2. Linear multivariate model (GEE).

This figure displays results of the linear multivariate model (GEE) of faecal E. coli retrieved from veal calves in Belgium (2011-2017).

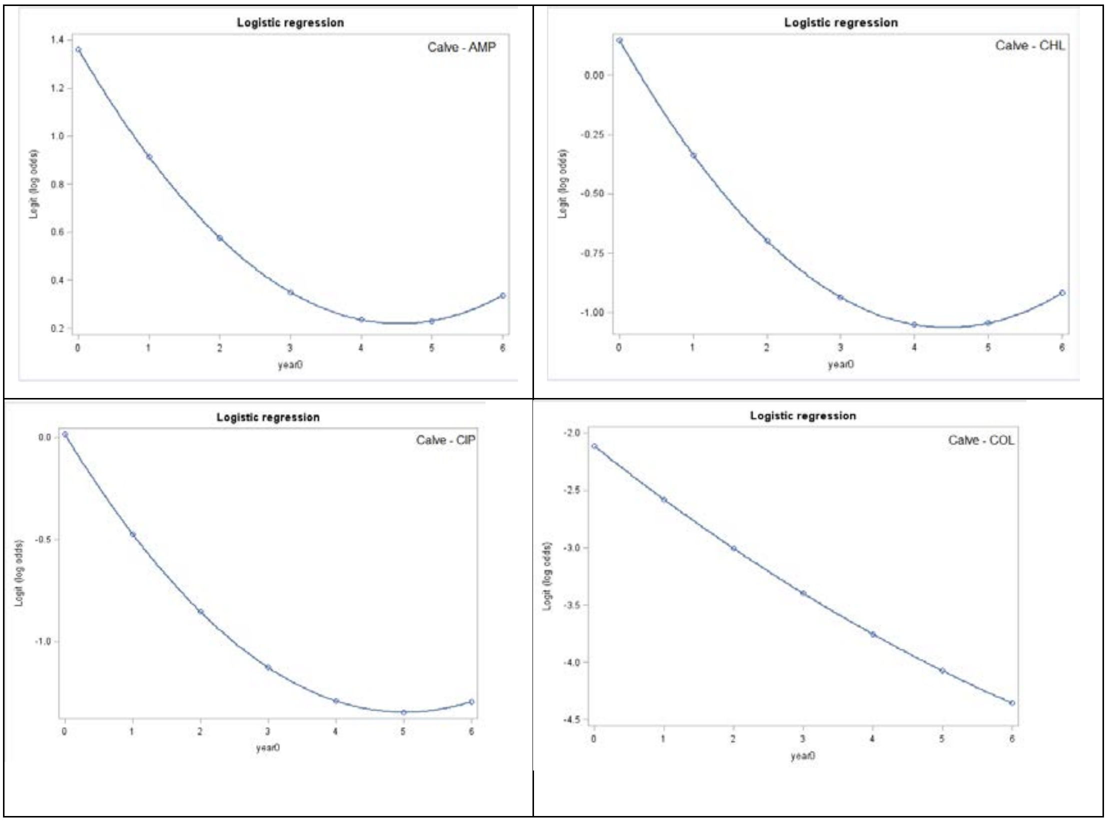
The detailed odds ratios obtained from the non-linear mixed multivariate model are shown in **table 2** and the log odds of the logistic regression are plotted in **figure 3**. Based on the non-linear mixed multivariate model we notice a constant significant decrease resistance (OR<1) for all substances from 2011 to 2014. However, OR are increasing in all substances and the decrease is considered as no longer significant from 2015 onwards for AMP, FOT, GEN, TAZ and from 2016 onwards for CHL, CIP, COL, SMX, TET and TMP. It can be noted that even if increases are not significant, in 2017, 9 substances present an odds ratio>1 and TMP increase in resistance is limit to be significant in 2017.

The only exception is NAL for which continuous significant decrease in resistance is noticed since 2011 (OR is <1 but however approaching OR=1 years after years).

Table 2. Results of the non-linear mixed multivariate model by antimicrobial substance and by years

Substance	OR1: year 2012 vs 2011			OR2: year 2013 vs 2012			OR3: year 2014 vs 2013			OR4: year 2015 vs 2014			OR5: year 2016 vs 2015			OR6: year 2017 vs 2016		
	Estimate	Lower limit	Upper limit	Estimate	Lower limit	Upper limit	Estimate	Lower limit	Upper limit	Estimate	Lower limit	Upper limit	Estimate	Lower limit	Upper limit	Estimate	Lower limit	Upper limit
AMP	**0,65**	0,51	0,79	**0,77**	0,70	0,83	**0,88**	0,83	0,94	**1,00**	0,86	1,13	**1,11**	0,90	1,33	**1,23**	0,92	1,53
CHL	**0,63**	0,51	0,76	**0,69**	0,57	0,81	**0,78**	0,70	0,86	**0,89**	0,83	0,95	**1,01**	0,88	1,13	**1,15**	0,91	1,38
CIP	**0,64**	0,50	0,77	**0,69**	0,56	0,81	**0,76**	0,68	0,84	**0,85**	0,78	0,91	**0,94**	0,82	1,07	**1,05**	0,82	1,27
COL	**0,64**	0,32	0,97	**0,65**	0,41	0,90	**0,67**	0,53	0,81	**0,69**	0,52	0,85	**0,70**	0,41	1,00	**0,72**	0,26	1,17
FOT	**0,56**	0,37	0,76	**0,63**	0,41	0,84	**0,76**	0,61	0,92	**0,93**	0,79	1,08	**1,14**	0,82	1,46	**1,39**	0,77	2,02
GEN	**0,68**	0,43	0,94	**0,73**	0,52	0,95	**0,81**	0,67	0,94	**0,89**	0,77	1,01	**0,98**	0,74	1,21	**1,07**	0,67	1,48
NAL	**0,74**	0,55	0,93	**0,75**	0,61	0,88	**0,76**	0,68	0,84	**0,77**	0,70	0,84	**0,78**	0,66	0,90	**0,79**	0,60	0,98
SMX	**0,62**	0,48	0,75	**0,67**	0,54	0,79	**0,76**	0,67	0,84	**0,86**	0,80	0,92	**0,97**	0,86	1,08	**1,10**	0,89	1,31
TAZ	**0,54**	0,37	0,72	**0,60**	0,40	0,80	**0,74**	0,60	0,88	**0,92**	0,77	1,06	**1,13**	0,82	1,44	**1,39**	0,78	2,01
TET	**0,74**	0,55	0,92	**0,78**	0,63	0,93	**0,83**	0,74	0,93	**0,89**	0,83	0,96	**0,96**	0,84	1,07	**1,03**	0,82	1,23
TMP	**0,56**	0,46	0,67	**0,62**	0,51	0,73	**0,73**	0,65	0,81	**0,87**	0,81	0,93	**1,03**	0,91	1,15	**1,22**	0,99	1,45

OR: odds ratio
Dark green: significant decrease; light green: non-significant decrease; orange: non-significant increase



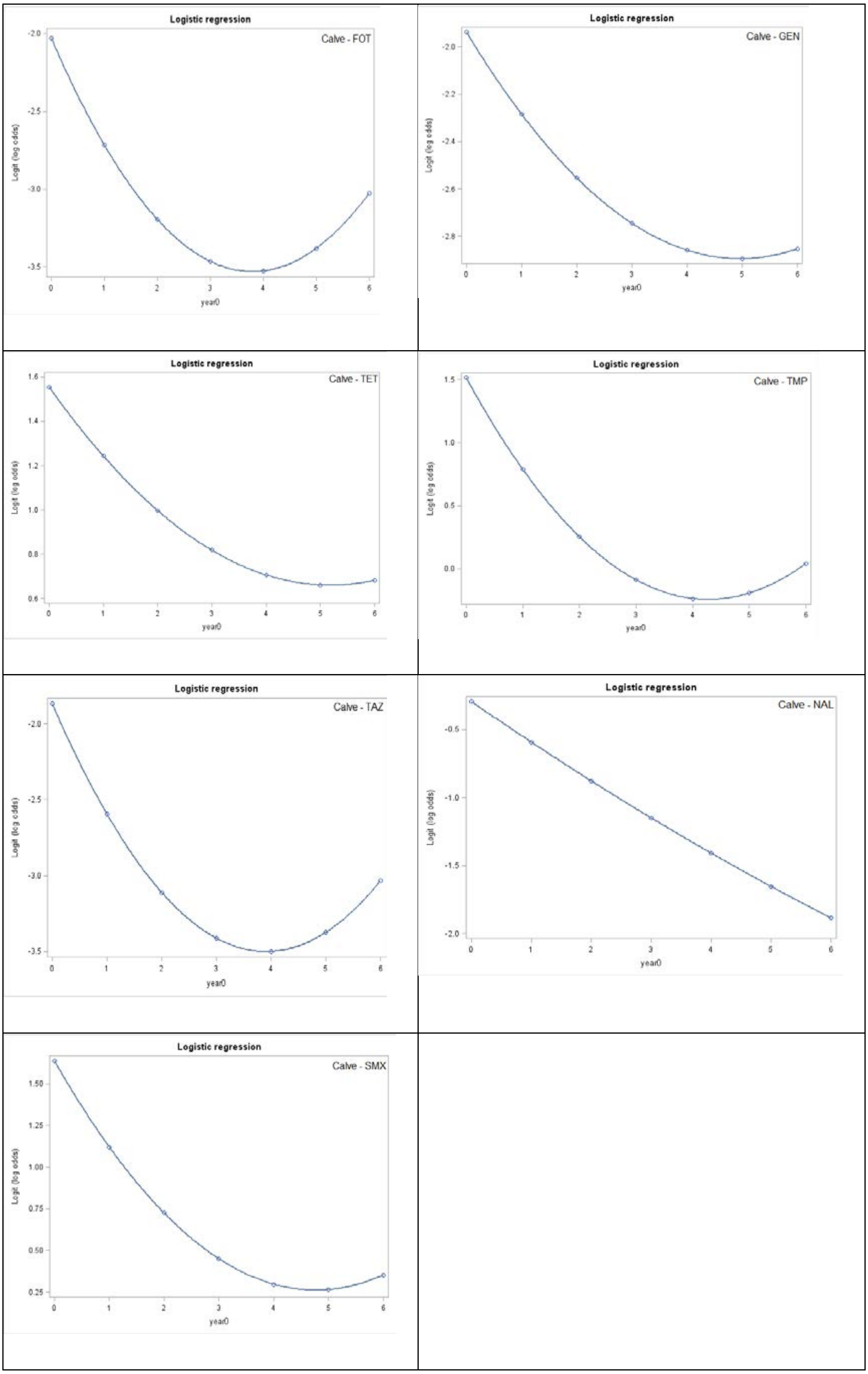
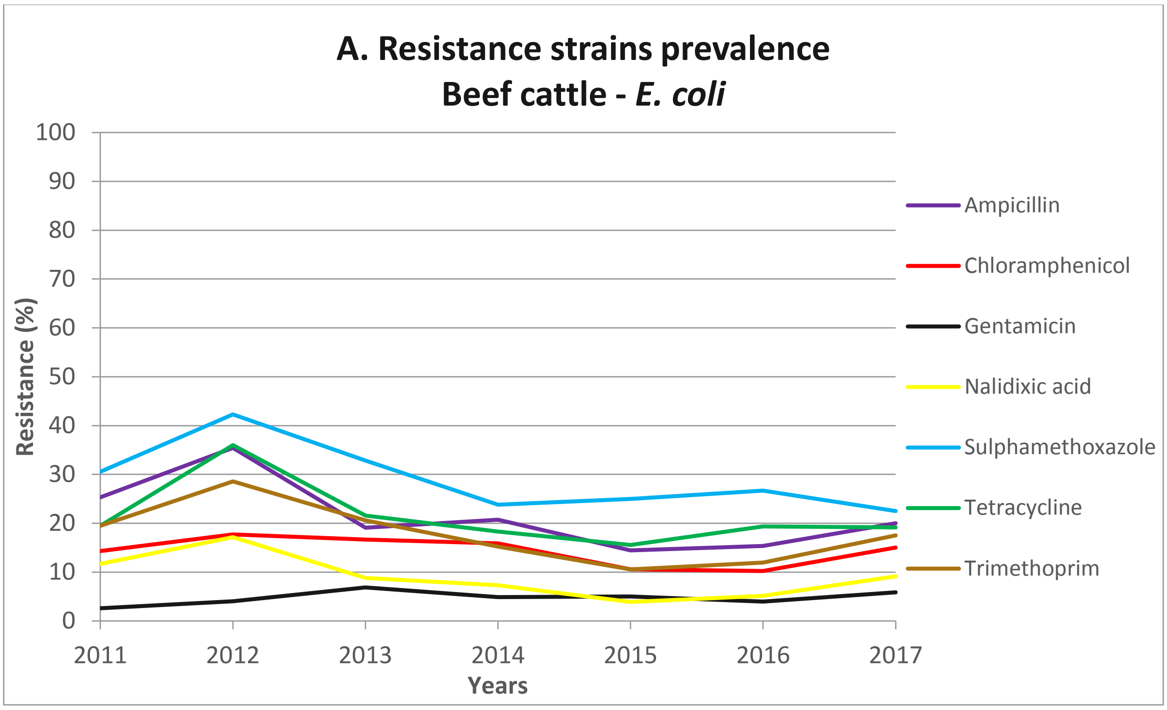


Figure 3. Logistic regression, by years.

Legend: year0: 0=2011; 1=2012; 2=2013; 3=2014; 4=2015 5=2016 6=2017.

b) Beef cattle: N= 154 (2011); 175 (2012); 204 (2013); 164 (2014); 180 (2015); 176 (2016))

Globally, significantly lower prevalences of resistance were observed in *E. coli* from beef cattle compared to veal calves. However, the highest resistance prevalences were observed against the same substances than for veal calves: AMP, SMX, TET and TMP (**figure 4a a**). SMX presents the highest prevalence of resistance but the prevalence in 2017 (22.50%) is the lowest ever observed in this category. Between 2016 and 2017, prevalence increased by >5% for CIP, FOT and TMP (**figure 4b**).



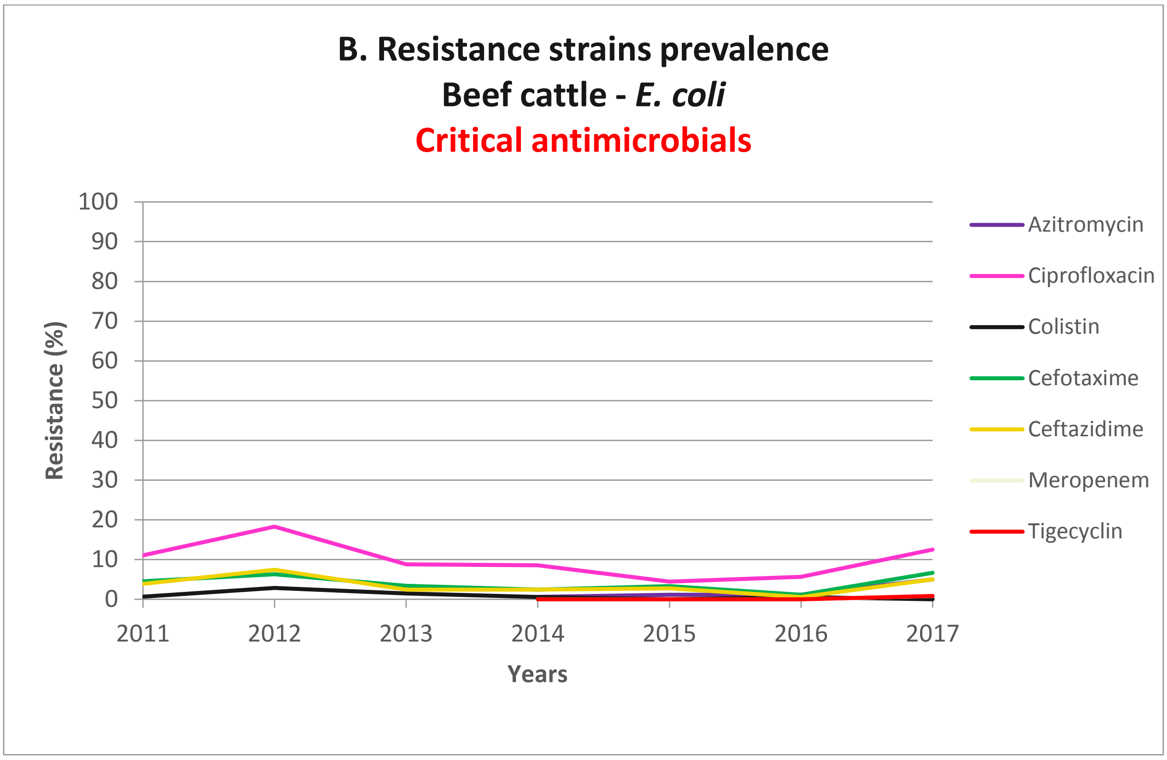


Figure 4a and 4b. Resistance strains prevalence: beef cattle

These figure describe the antimicrobial susceptibility trends of faecal E. coli retrieved from beef cattle in Belgium (2011-2017).

Based on the results of the linear multivariate model (GEE), the probability to be resistant decrease significantly over time for all tested substances except for CHL, FOT, TAZ and GEN (**figure 5**).

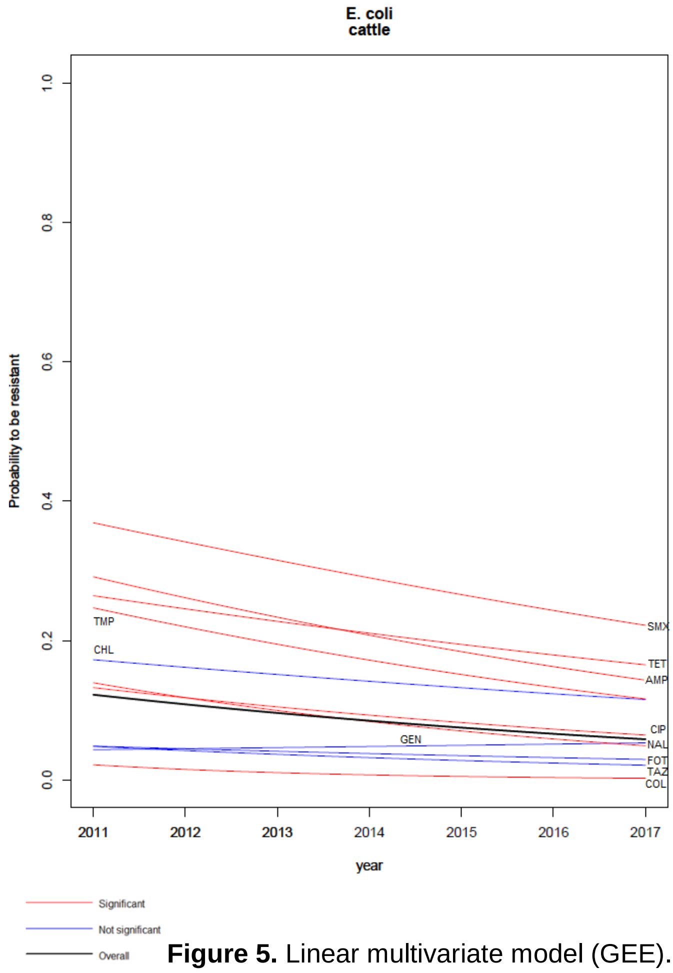


Figure 5. Linear multivariate model (GEE).

This figure displays results of the linear multivariate model (GEE) of faecal E. coli retrieved from beef cattle in Belgium (2011-2017).

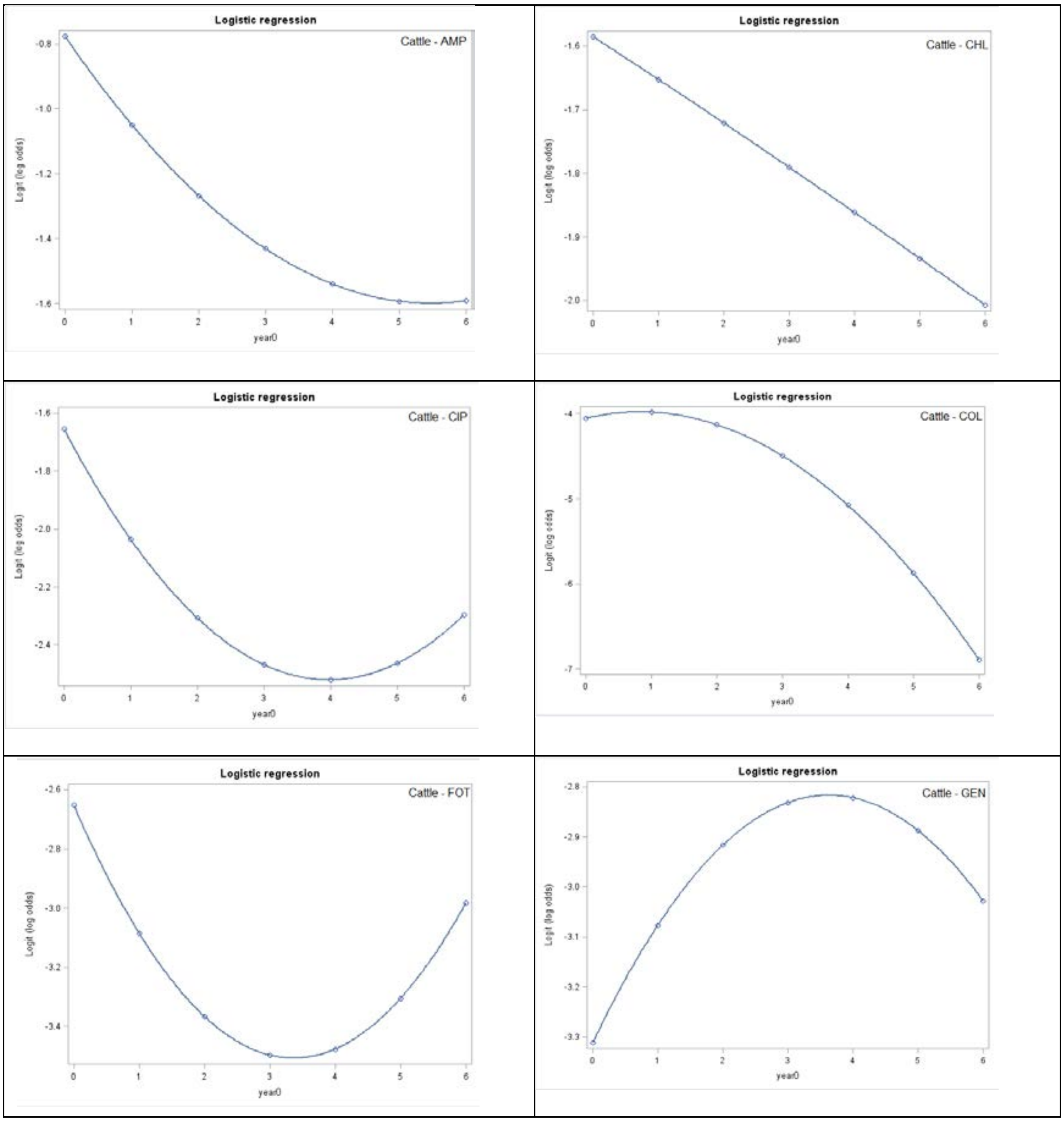
The detailed odds ratios obtained from the non-linear mixed multivariate model are shown in **table 3** and the log odds of the logistic regression are plotted in **figure 6**. Based on the non-linear mixed multivariate model we notice a constant significant decrease in resistance (OR<1) for FOT (2011-2013), AMP, CIP, NAL, TAZ (2011-2014) and for TMP (2011-2015) but, except for SMX, this decrease is followed by a continuous non-significant increase in resistance (OR>1). COL is the only substance that shows a continuous decrease in resistance since 2011 (OR<1 since 2014) and this decrease is significant for COL from 2015. However, prevalence for COL was already is really low. We also observe that OR of GEN are <1 since 2016, but not significant.

Table 3. Results of the non-linear mixed multivariate model by antimicrobial substance and by years

Substance	OR1: year 2012 vs 2011			OR2: year 2013 vs 2012			OR3: year 2014 vs 2013			OR4: year 2015 vs 2014			OR5: year 2016 vs 2015			OR6: year 2017 vs 2016		
	Estimate	Lower limit	Upper limit	Estimate	Lower limit	Upper limit	Estimate	Lower limit	Upper limit	Estimate	Lower limit	Upper limit	Estimate	Lower limit	Upper limit	Estimate	Lower limit	Upper limit
AMP	**0,77**	0,62	0,92	**0,82**	0,75	0,90	**0,88**	0,81	0,94	**0,93**	0,80	1,06	**0,98**	0,77	1,20	**1,04**	0,74	1,33
CHL	**0,94**	0,71	1,18	**0,94**	0,79	1,09	**0,93**	0,85	1,02	**0,93**	0,83	1,03	**0,92**	0,75	1,10	**0,92**	0,66	1,18
CIP	**0,71**	0,54	0,88	**0,77**	0,63	0,91	**0,85**	0,76	0,94	**0,95**	0,83	1,06	**1,05**	0,82	1,28	**1,17**	0,79	1,55
COL	**1,78**	- 0,73	4,29	**1,02**	0,39	1,66	**0,65**	0,30	1,01	**0,42**	0,00	0,83	**0,27**	- 0,15	0,68	**0,17**	- 0,20	0,54
FOT	**0,68**	0,45	0,92	**0,76**	0,55	0,97	**0,88**	0,74	1,02	**1,02**	0,84	1,20	**1,18**	0,81	1,55	**1,36**	0,71	2,01
GEN	**1,25**	0,65	1,86	**1,16**	0,82	1,50	**1,08**	0,89	1,26	**1,00**	0,83	1,18	**0,94**	0,66	1,21	**0,87**	0,48	1,26
NAL	**0,70**	0,52	0,87	**0,74**	0,61	0,88	**0,81**	0,72	0,90	**0,88**	0,76	1,01	**0,96**	0,73	1,19	**1,05**	0,68	1,42

SMX	**0,91**	0,73	1,08	**0,90**	0,79	1,02	**0,90**	0,83	0,96	**0,89**	0,82	0,96	**0,88**	0,76	1,01	**0,88**	0,69	1,06
TAZ	**0,70**	0,43	0,96	**0,76**	0,53	0,98	**0,84**	0,70	0,99	**0,94**	0,75	1,14	**1,05**	0,67	1,43	**1,17**	0,54	1,79
TET	**0,88**	0,70	1,07	**0,89**	0,77	1,02	**0,90**	0,83	0,98	**0,92**	0,83	1,00	**0,93**	0,78	1,07	**0,94**	0,72	1,16
TMP	**0,79**	0,63	0,96	**0,82**	0,70	0,94	**0,85**	0,78	0,92	**0,89**	0,80	0,98	**0,92**	0,76	1,08	**0,96**	0,71	1,21

OR: odds ratio
Dark green: significant decrease; light green: non-significant decrease; orange: non-significant increase



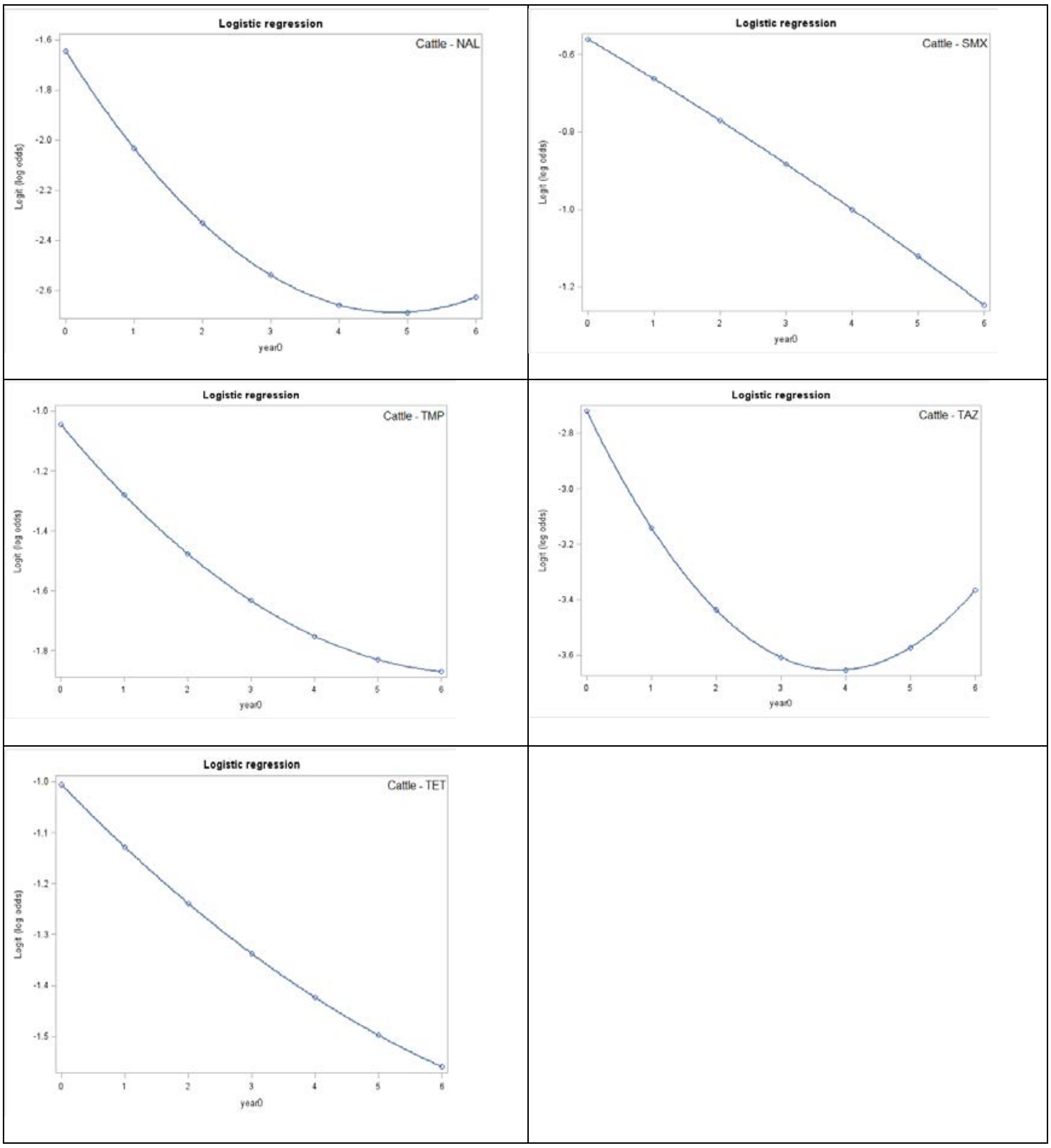
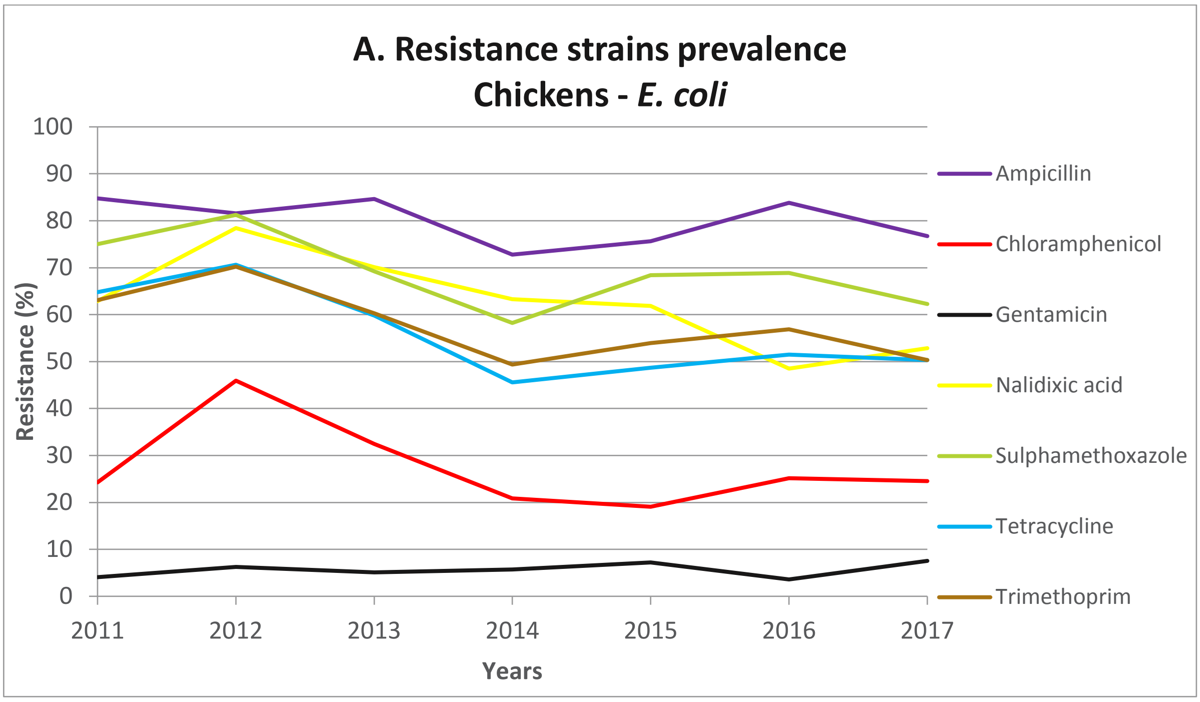


Figure 6. Logistic regression, by years.

Legend: year0: 0=2011; 1=2012; 2=2013; 3=2014; 4=2015; 5=2016; 6=2017.

c) Broiler Chickens (N= 420 (2011); 320 (2012); 234 (2013); 158 (2014); 152 (2015); 167 (2016); 159 (2017))

A high prevalence of resistance was observed for broiler chickens with values ≥ 50% for the seven consecutive years for AMP, CIP and SMX and with values ≥ 50% for six years for NAL, TMP (**figures 7a and 7b**). Prevalence of resistance increased from 2016 to 2017 by 9.95% and by 8.63% for FOT and TAZ respectively.



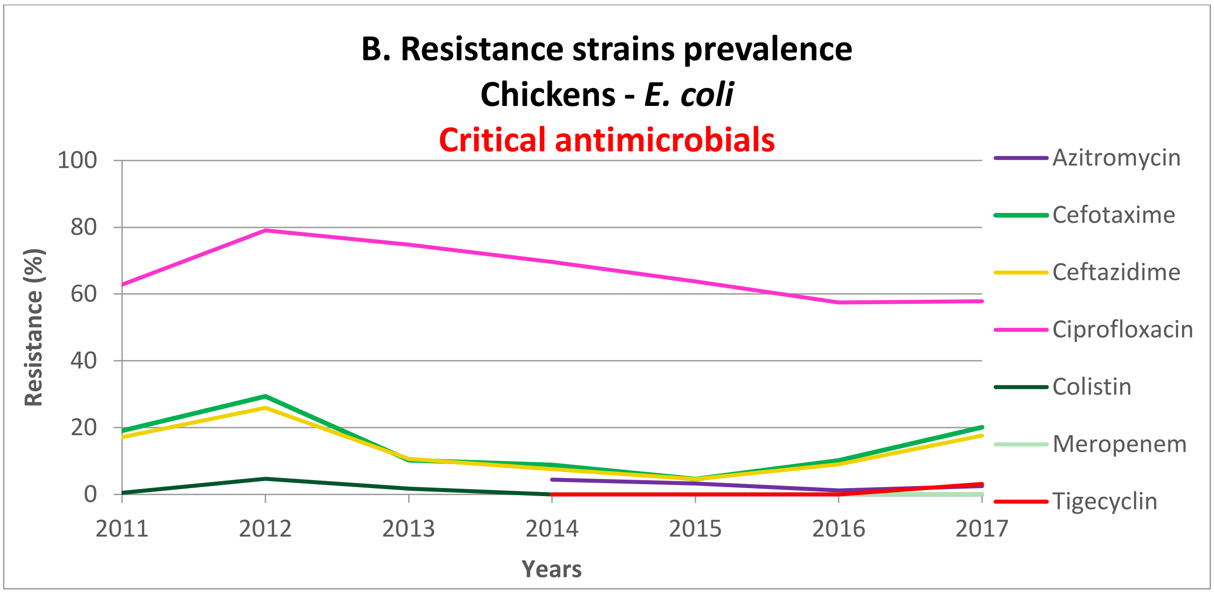


Figure 7a and 7b. Resistance strains prevalence: chickens.

These figures describe the antimicrobial susceptibility trends of faecal E. coli retrieved from chickens in Belgium (2011-2017).

Based on the results of the linear multivariate model (GEE), the probability to be resistant significantly decrease over time for all tested substances except for GEN (**figure 8**). AMP, CIP, SMX, NAL, TMP, TET, substances with high levels of resistance, showed a statistically significant decrease.

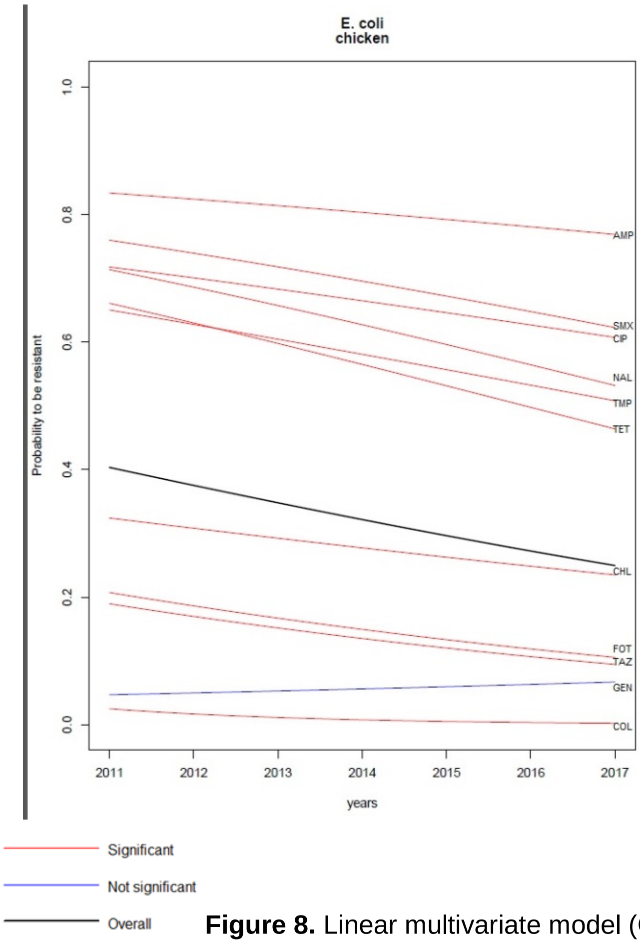


Figure 8. Linear multivariate model (GEE)

This figure displays results of the linear multivariate model (GEE) of faecal E. coli retrieved from chickens in Belgium (2011-2017).

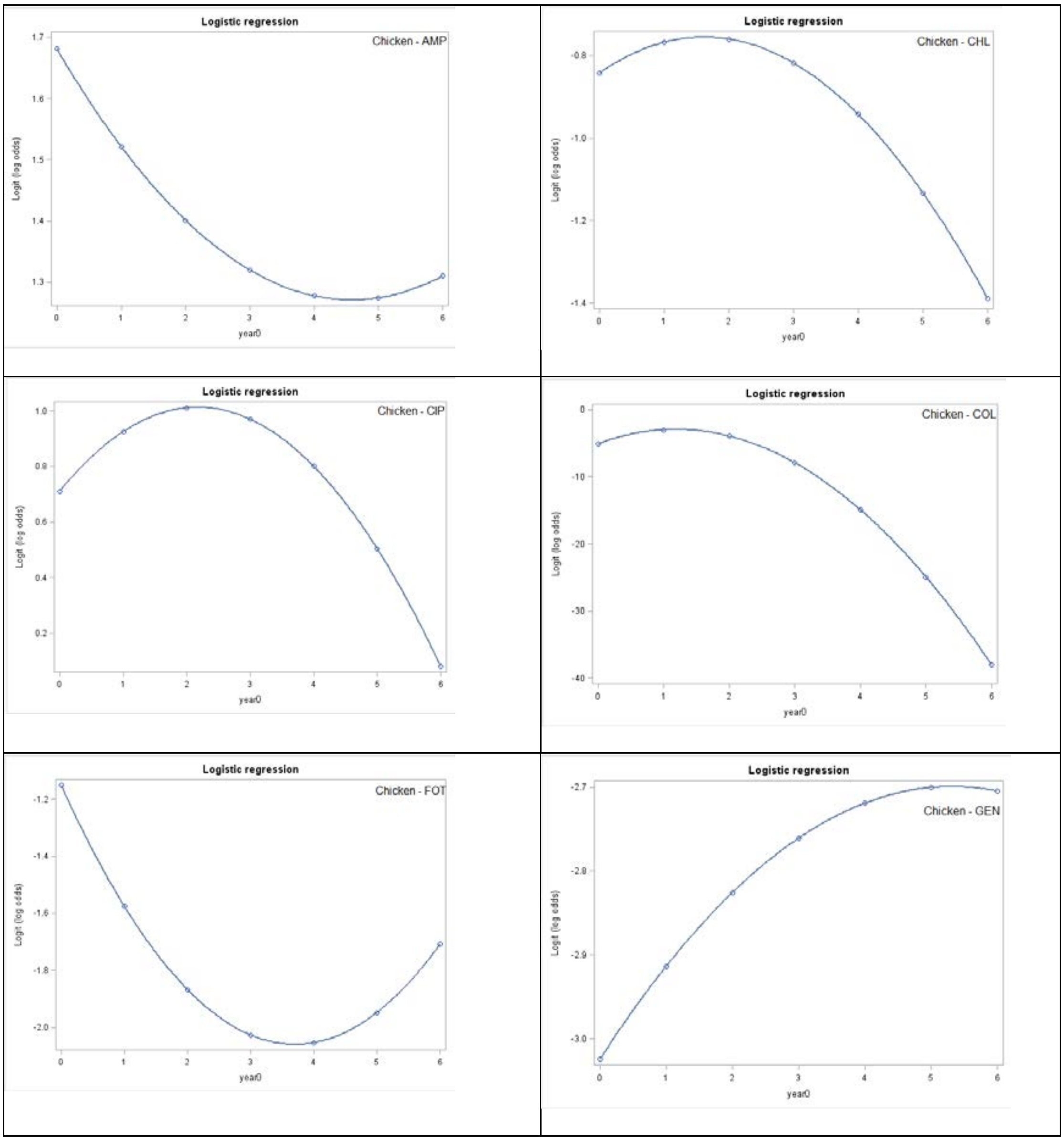
The detailed odds ratios obtained from the non-linear mixed multivariate model are shown in **table 4** and the log odds of the logistic regression are plotted in **figure 9**. An increasing trend was previously detected by NL models for CIP in 2012 but afterward, a constant decrease of resistance has been observed, significant since the last years. Globally, whatever the NL model used, there is a decreasing trend in resistance in CHL, CIP (significant), COL (significant), NAL (significant). It should be mentioned that AMP, FOT and TAZ present odds ratio >1 by both NL models in 2016 and in 2017, however not significant.

Table 4: Results of the non-linear mixed multivariate model by antimicrobial substance and by years

Substance	OR1: year 2012 vs 2011			OR2: year 2013 vs 2012			OR3: year 2014 vs 2013			OR4: year 2015 vs 2014			OR5: year 2016 vs 2015			OR6: year 2017 vs 2016		
	Estimate	Lower limit	Upper limit	Estimate	Lower limit	Upper limit	Estimate	Lower limit	Upper limit	Estimate	Lower limit	Upper limit	Estimate	Lower limit	Upper limit	Lower limit	Upper limit	Lower limit
AMP	**0,85**	0,71	1,00	**0,89**	0,81	0,98	**0,93**	0,88	0,99	**0,97**	0,88	1,07	**1,02**	0,85	1,18	**1,06**	0,82	1,29
CHL	**1,08**	0,90	1,26	**1,01**	0,91	1,11	**0,94**	0,89	1,00	**0,88**	0,82	0,95	**0,83**	0,72	0,93	**0,77**	0,62	0,92
CIP	**1,25**	1,04	1,47	**1,09**	0,98	1,19	**0,96**	0,91	1,01	**0,84**	0,79	0,90	**0,74**	0,66	0,83	**0,65**	0,54	0,77
COL	**52,63**	-74,92	180,18	**0,36**	-0,02	0,74	**0,01**	-0,02	0,05	**0,00**	0,00	0,00	**0,00**	0,00	0,00	**0,00**	0,00	0,01
FOT	**0,68**	0,58	0,78	**0,75**	0,66	0,83	**0,85**	0,79	0,91	**0,97**	0,89	1,06	**1,11**	0,94	1,28	**1,27**	0,97	1,56
GEN	**1,14**	0,76	1,52	**1,11**	0,88	1,33	**1,07**	0,95	1,20	**1,04**	0,90	1,18	**1,01**	0,77	1,24	**0,98**	0,63	1,32
NAL	**1,11**	0,93	1,29	**1,00**	0,91	1,10	**0,91**	0,86	0,95	**0,82**	0,77	0,87	**0,74**	0,66	0,83	**0,67**	0,56	0,79
SMX	**0,85**	0,72	0,98	**0,87**	0,78	0,96	**0,89**	0,84	0,94	**0,91**	0,85	0,97	**0,93**	0,82	1,04	**0,95**	0,79	1,12
TAZ	**0,69**	0,58	0,80	**0,76**	0,66	0,85	**0,85**	0,79	0,91	**0,96**	0,87	1,05	**1,08**	0,90	1,26	**1,22**	0,92	1,51

TET	**0,81**	0,70	0,92	**0,84**	0,76	0,91	**0,86**	0,82	0,91	**0,89**	0,84	0,95	**0,92**	0,82	1,02	**0,95**	0,79	1,11
TMP	**0,91**	0,78	1,04	**0,91**	0,82	0,99	**0,91**	0,86	0,95	**0,90**	0,85	0,96	**0,90**	0,80	1,00	**0,90**	0,75	1,05

OR: odds ratio
Dark green: significant decrease; light green: non-significant decrease; orange: non-significant increase; red: significant increase



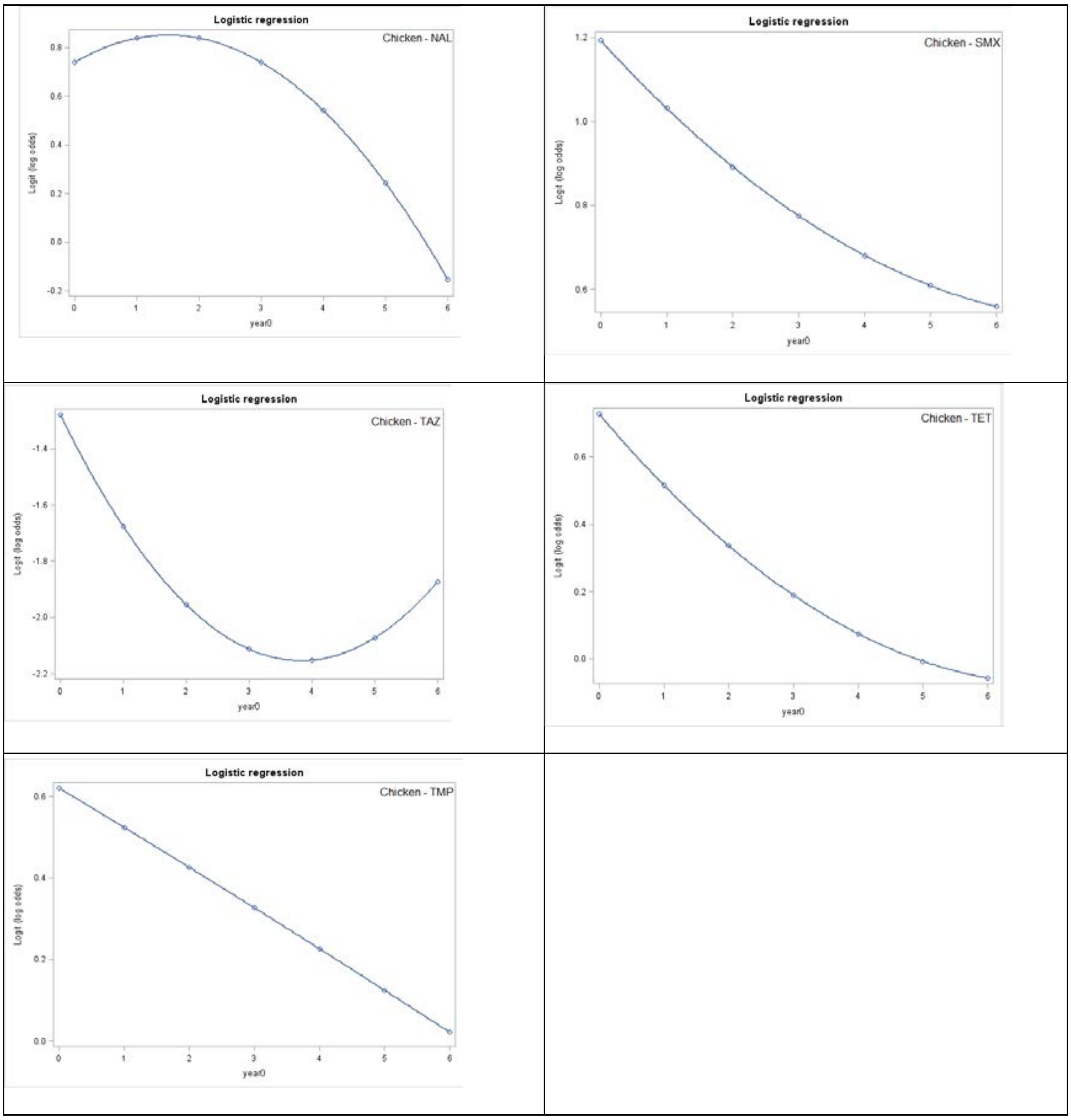
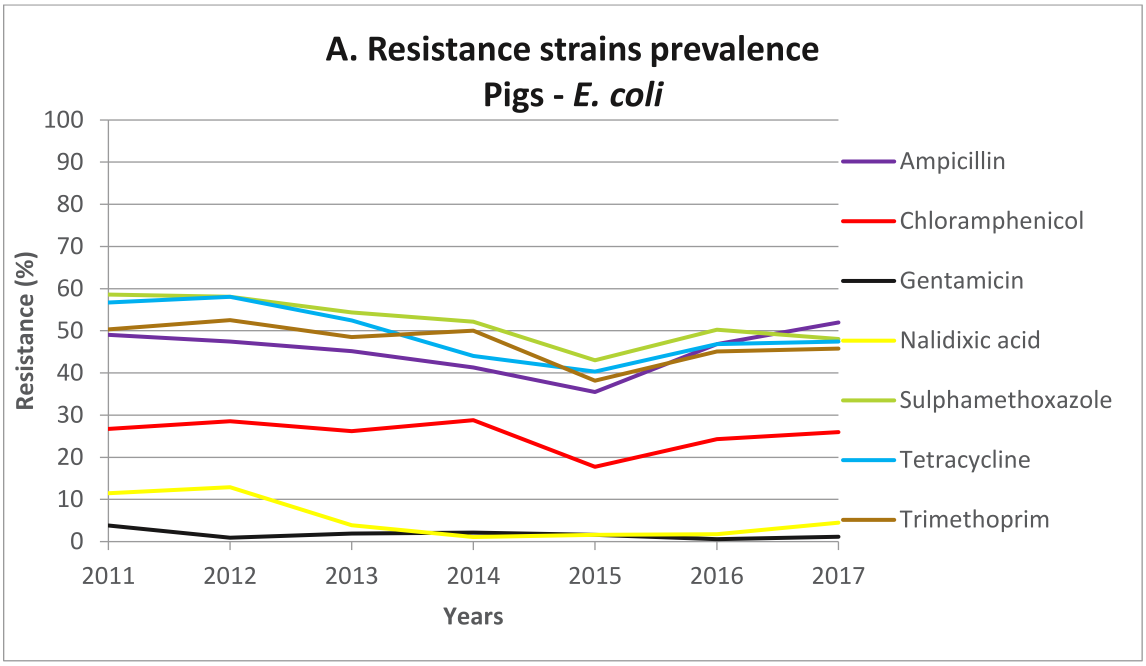


Figure 9. Logistic regression, by years.

Legend: year0: 0=2011; 1=2012; 2=2013; 3=2014; 4=2015; 5=2016; 6=2017.

d) Pigs: (N= 157 (2011) ; 217 (2012) ; 206 (2013); 184 (2014); 186 (2015); 173 (2016); 177 (2017))

The prevalences of resistance for SMX, TET, AMP, GEN was above 40% during years (2011-2014/2016-2017) and during the seven consecutive years for TET and SMX (**figure 10a**). AMP is in 2017 for the first time the antimicrobial with the highest prevalence in pigs (4^{th} from 2011 to 2015). Prevalences for FOT and TAZ increased by +8.42% and by +7.86% respectively between 2016 and 2017. For COL and GEN prevalences of these two substances are very low (<4%) (**figure 10b)**.



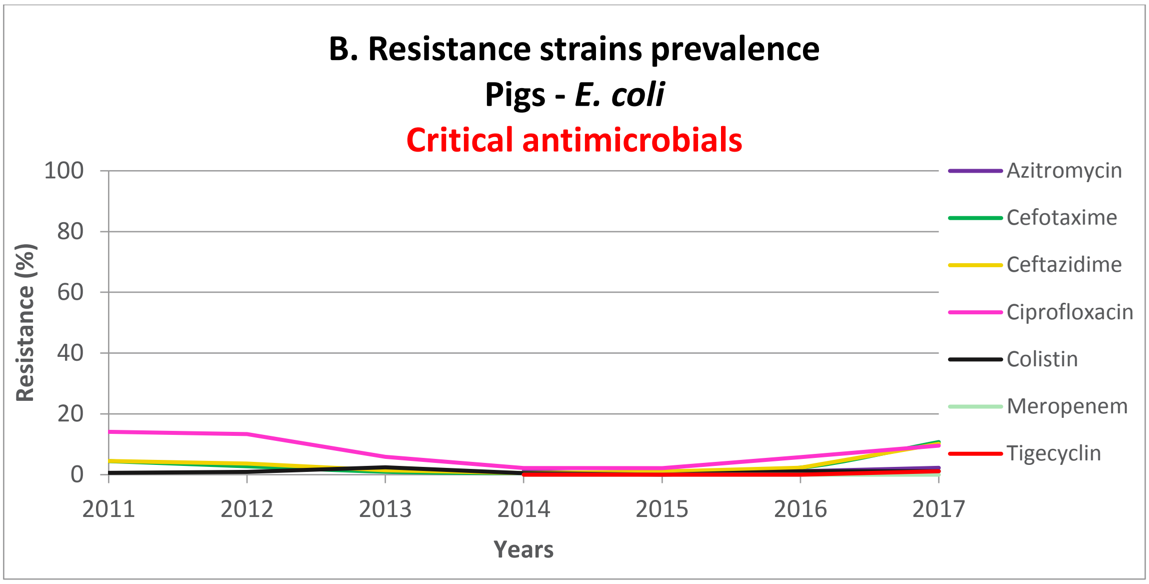


Figure 10a and 10b. Resistance strains prevalence: pigs

These figures describe the antimicrobial susceptibility trends of faecal *E. coli* retrieved from pigs in, Belgium (2011-2017).

Based on the results of the linear multivariate model (GEE) (**figure 11**), the probability to be resistant decrease significantly over time for SMX, TMP, TET, CIP, NAL.

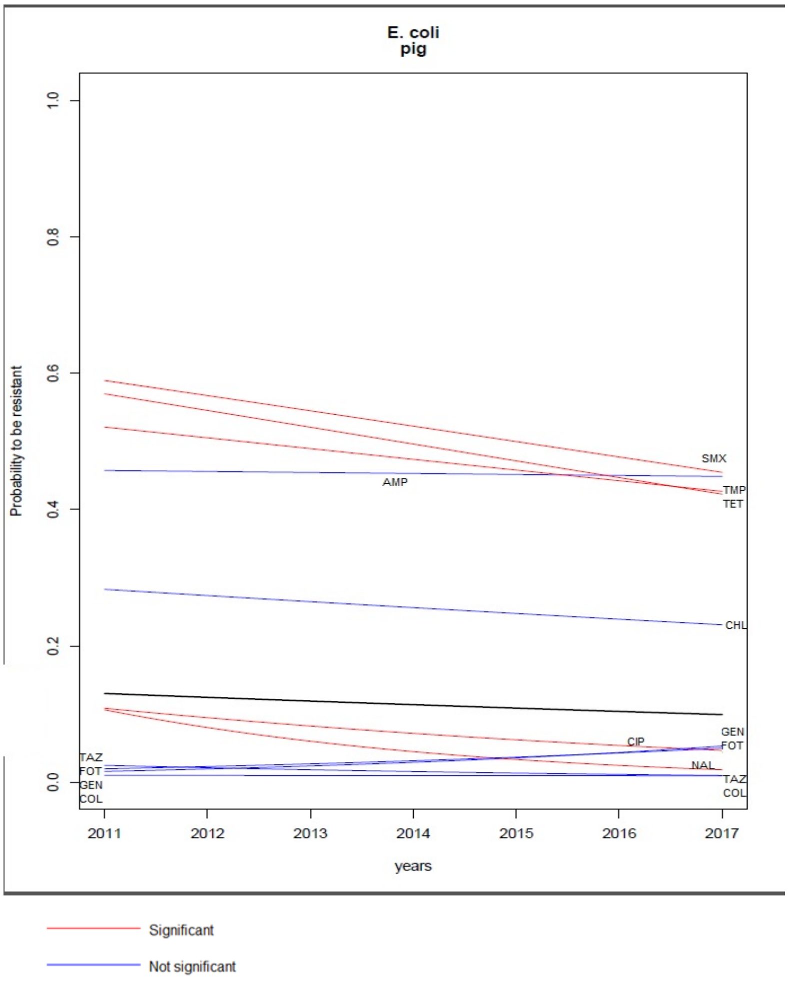


Figure 11. Linear multivariate model (GEE)

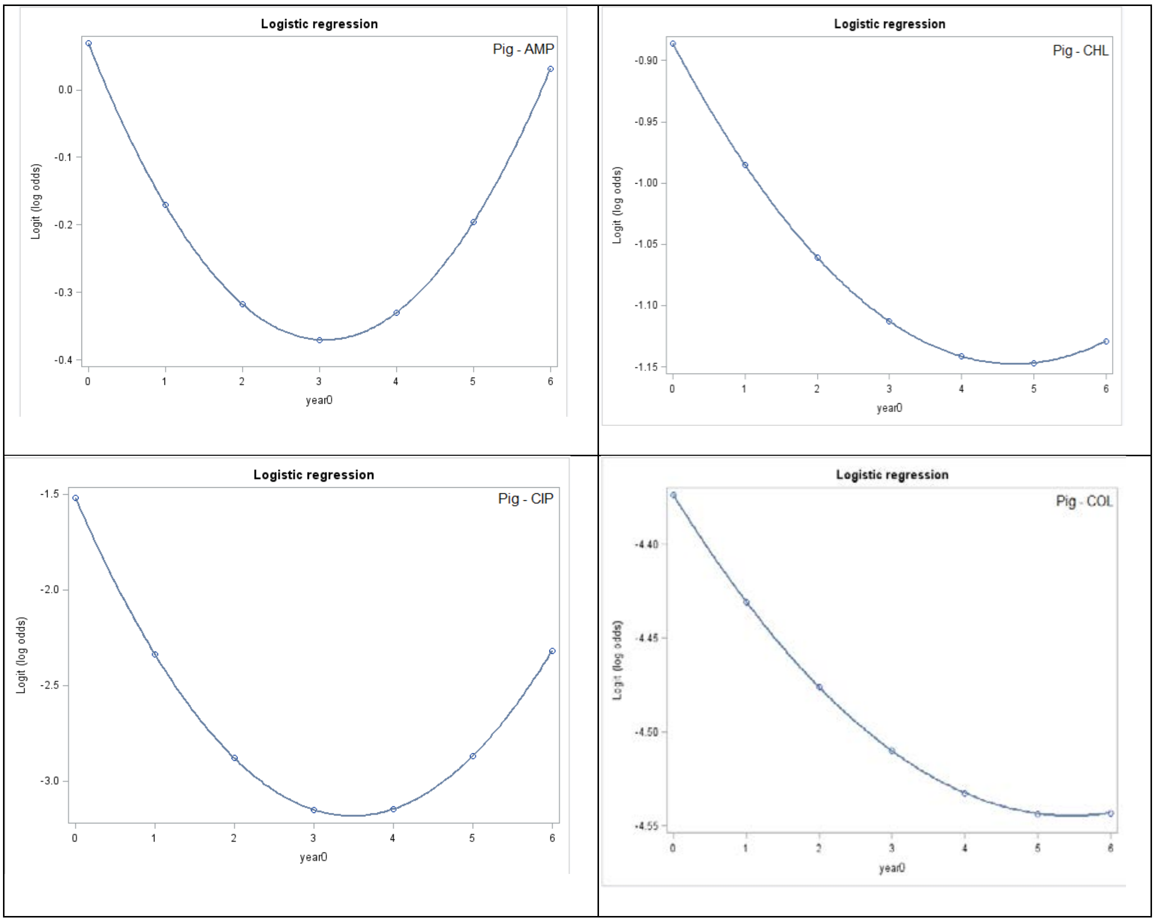
This figure displays results of the linear multivariate model (GEE) of faecal *E. coli* retrieved from pigs in Belgium (2011-2017).

The detailed odds ratios obtained from the non-linear mixed multivariate model are shown in **table 5** and the log odds of the logistic regression are plotted in **figure 12**. Based on the non-linear multivariate model we notice that, except for GEN in 2013, there is a constant increase in resistance. Whatever the NL model used, this increase is significant for AMP, CIP, FOT and TAZ beginning in 2016 or even before.

Table 5: Results of the non-linear mixed multivariate model by antimicrobial substance and by years

Substance	OR1: year 2012 vs 2011			OR2: year 2013 vs 2012			OR3: year 2014 vs 2013			OR4: year 2015 vs 2014			OR5: year 2016 vs 2015			OR6: year 2017 vs 2016		
	Estimate	Lower limit	Upper limit	Estimate	Lower limit	Upper limit	Estimate	Lower limit	Upper limit	Estimate	Lower limit	Upper limit	Estimate	Lower limit	Upper limit	Estimate	Lower limit	Upper limit
AMP	**0,80**	0,68	0,93	**0,89**	0,83	0,96	**0,99**	0,94	1,03	**1,08**	0,99	1,17	**1,17**	1,02	1,32	**1,26**	1,05	1,47
CHL	**0,92**	0,74	1,09	**0,93**	0,81	1,05	**0,95**	0,88	1,02	**0,97**	0,90	1,04	**0,99**	0,87	1,12	**1,01**	0,81	1,21
CIP	**0,52**	0,44	0,60	**0,58**	0,47	0,69	**0,76**	0,68	0,84	**1,00**	0,89	1,12	**1,32**	1,04	1,60	**1,74**	1,18	2,30
COL	**1,10**	0,08	2,12	**1,05**	0,44	1,66	**1,00**	0,66	1,34	**0,95**	0,63	1,28	**0,91**	0,38	1,44	**0,87**	0,09	1,64
FOT	**0,50**	0,44	0,57	**0,54**	0,35	0,72	**0,88**	0,72	1,04	**1,46**	1,24	1,68	**2,40**	1,66	3,14	**3,96**	1,98	5,93
GEN	**0,83**	0,33	1,33	**0,84**	0,50	1,17	**0,85**	0,65	1,05	**0,85**	0,61	1,10	**0,86**	0,44	1,29	**0,87**	0,24	1,51
NAL	**0,51**	0,41	0,61	**0,55**	0,43	0,67	**0,68**	0,59	0,77	**0,84**	0,70	0,98	**1,03**	0,73	1,33	**1,27**	0,73	1,82
SMX	**0,86**	0,71	1,00	**0,88**	0,78	0,98	**0,91**	0,84	0,97	**0,93**	0,87	0,99	**0,96**	0,85	1,06	**0,99**	0,82	1,15
TAZ	**0,51**	0,43	0,59	**0,56**	0,38	0,75	**0,88**	0,73	1,02	**1,37**	1,17	1,57	**2,13**	1,51	2,75	**3,32**	1,76	4,89
TET	**0,78**	0,66	0,90	**0,83**	0,73	0,92	**0,88**	0,82	0,94	**0,94**	0,88	1,00	**1,00**	0,89	1,11	**1,07**	0,89	1,25
TMP	**0,85**	0,71	0,99	**0,88**	0,78	0,98	**0,91**	0,85	0,97	**0,95**	0,89	1,01	**0,99**	0,88	1,10	**1,03**	0,85	1,20

OR: odds ratio
Dark green: significant decrease; light green: non-significant decrease; orange: non-significant increase; red significant increase



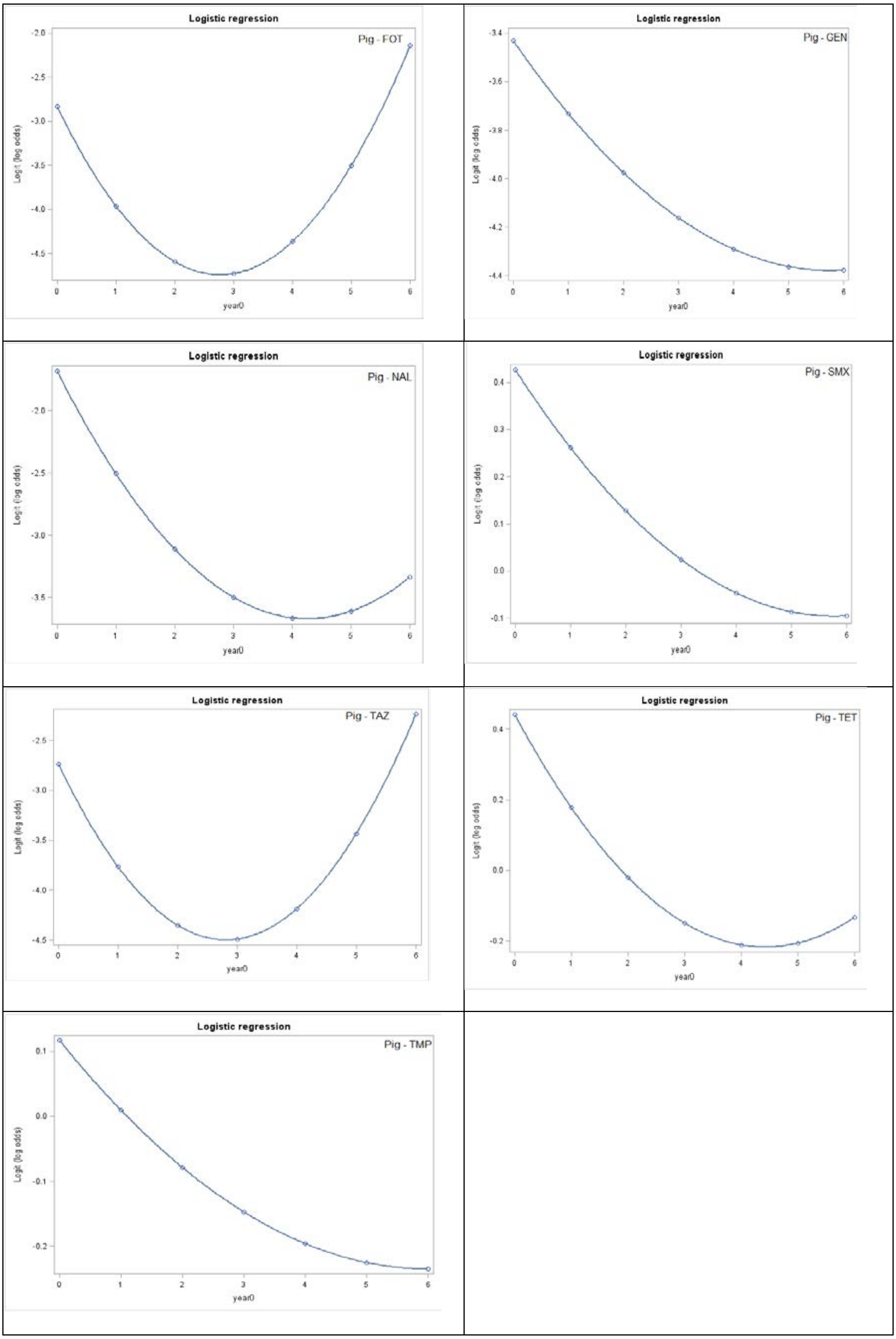


Figure 12: logistic regression, by years.

Legend: year0: 0=2011; 1=2012; 2=2013; 3=2014; 4=2015; 5=2016; 6=2017.

E. Multi-resistance

➢ Prevalence of multi-resistance

The proportion of multi-resistant strains (= strains resistant to at least three antimicrobials) was very high for broiler chickens (>62%) and high for veal calves (>50%) during the seven consecutive years (**Table 6** and **Figure 13**). Except in chickens, multi-resistance has increased since 2015. In beef, this increase happened in 2017 after four consecutive years of decrease (2013-2016). For the third year, multi-resistance to 9 different antimicrobial classes is observed in veal calves in 2017 (1% of strains).

Figure 14 displays the distribution of multi-resistance patterns per animal category (i.e, number of isolates resistant to 0, 1….9 of the antimicrobial classes tested).
25.95%, 72.50%, 11.32%, 27.12%, of, respectively, meat calves, young bovine, chicken and pig isolates, were fully susceptible (=no resistance) in 2017 to all tested antimicrobials.

Table 6: proportion of multi-resistant strains (%) (+95% confidence interval)

	Veal calves	Beef cattle	Chickens	Pigs
2011	**70.59** (54.45-86.73)	**24.68** (17.79-31.56)	**77.86** (73.87-81.84)	**53.50** (45.62-61.39)
2012	**72.93** (66.39-79.46)	**32.57** (25.56-39.58)	**81.88** (77.63-86.12)	**53.92** (47.23-60.6)
2013	**66.83** (60.28-73.38)	**23.04 (**17.21-28.87)	**76.92** (71.48-82.36)	**48.54** (41.66-55.43)
2014	**56.38** (49.23-63.54)	**20.73** (14.46-27)	**62.03** (54.37-69.68)	**47.83** (40.54-55.11)
2015	**51.02** (43.96-58.08)	**16.67** (11.17-22.16)	**70.39** (63.05-77.73)	**36.56** (29.57-43.54**)**
2016	**58.05** (50.64-65.45)	**15.91** (10.45-21.37)	**68.86** (61.77-75.96)	**45.09** (37.60-52.57)
2017	**56.76** (49.55-63.96)	**22.50** (14.92-30.08)	**67.30** (59.92-74.67)	**48.02** (40.59-55.45)

This table shows the proportion (%) and 95% confidence interval of multi-resistance from faecal *E. coli* retrieved from veal calves, beef cattle, chickens and pigs in Belgium (2011-2017).

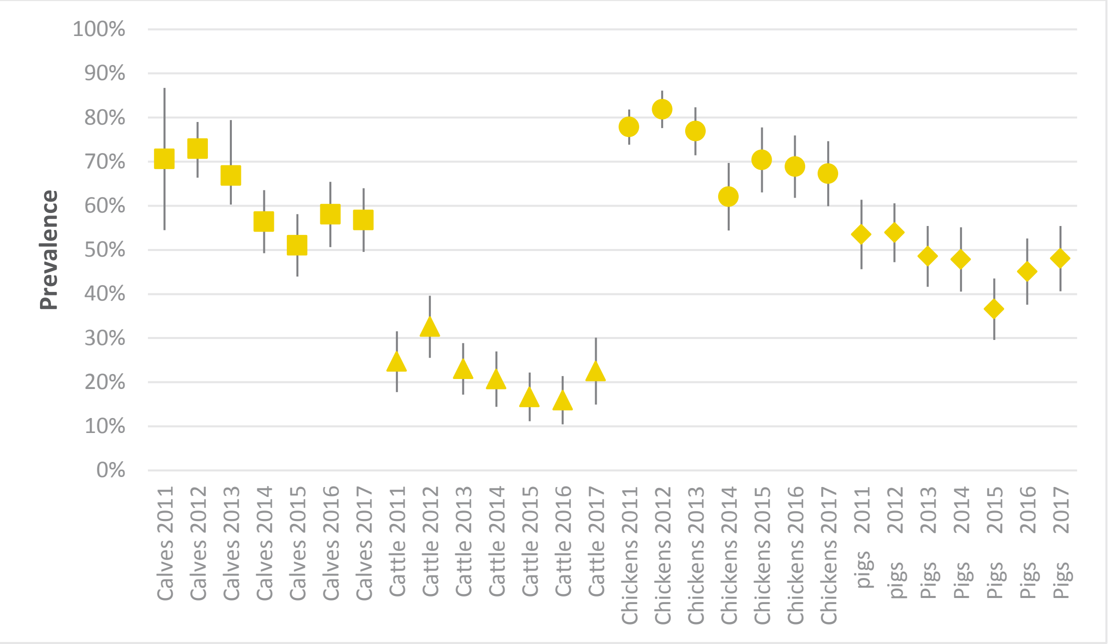
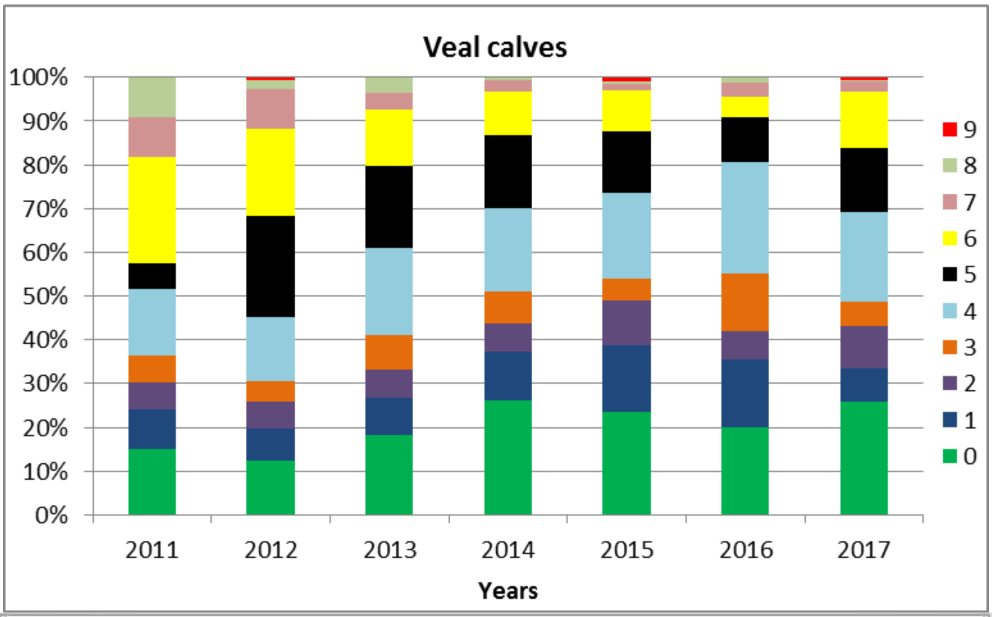
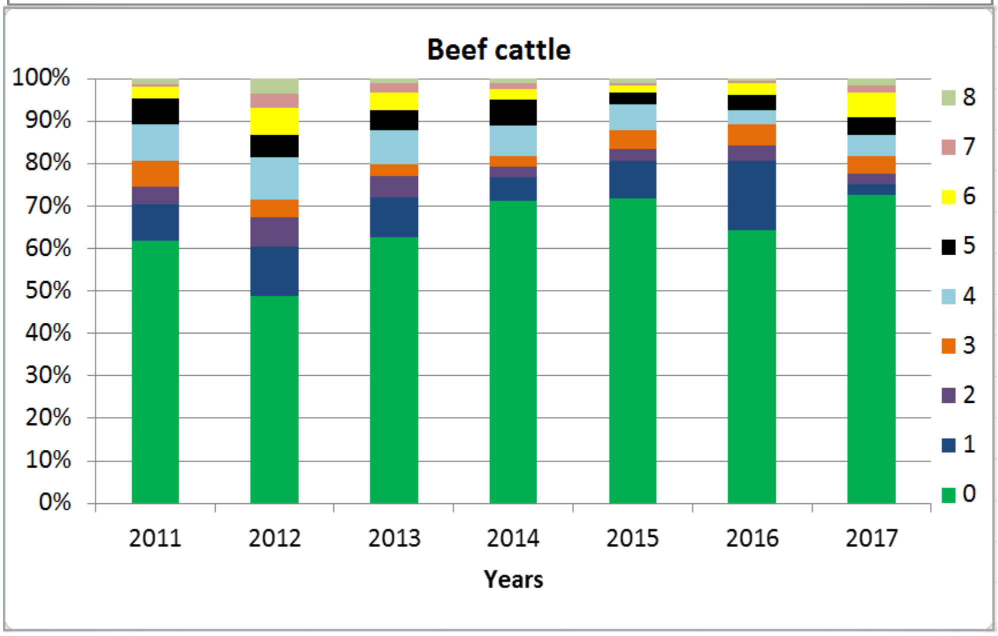


Figure 13. Proportion of multi-resistant strains (+95% CI).
This figure graphically represents multi-resistance prevalence, for veal calves, beef cattle, chickens and pigs and by years (same data displayed on table 6).


Veal calves
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
2011
2012
2013
2014
2015
2016
2017
Years
9
8
7
6
5
4
3
2
1
0


Beef cattle
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
2011
2012
2013
2014
2015
2016
2017
Years
8
7
6
5
4
3
2
1
0

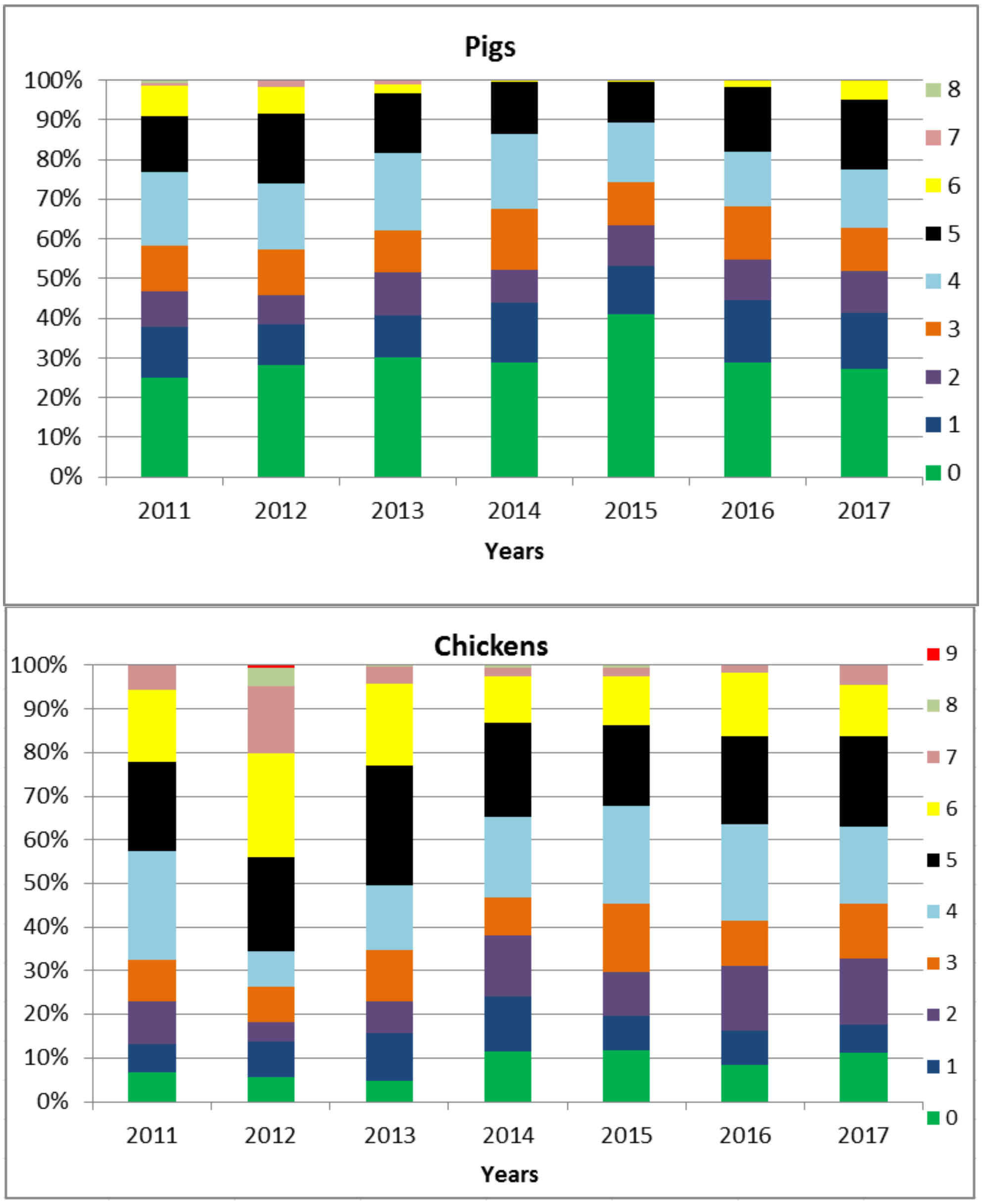


Figure 14. Distribution of multi-resistance patterns (%) per animal category and by years (2011-2017). 0= fully sensitive to 9= resistant to 9 different antimicrobials classes.

Table 7 and 8 present the OR (the ratio of the odds for a one-unit increase in the time) for multi-resistance obtained from the linear and non-linear models, respectively. For all species, significant decreases in multi-resistance were observed from 2011 but OR and 95%CI are progressively increasing and approaching 1 (non-significant) over the last few years.
Since 2016, no significant decreasing trend has been observed by logistic regression in any livestock species under investigation.

Table 7. Ratio of the odds and confidence intervals for multi-resistance obtained from the linear model (2011-2017), by species category.

Species	OR	95%CI
Veal calves	0.870	0.813-0.931
Beef cattle	0.891	0.828-0.959
Chickens	0.892	0.845-0.942

Pigs	0.934	0.884-0.988

OR= odds ratio; 95%CI= 95% confidence intervals

Table 8: Ratio of the odds and confidence intervals regarding to probability to be multi-resistant (logistic regression, year by year)

Years compared	Veal calves	Beef cattle	Chickens	Pig
2012 vs 2011	1.263 (0.561-2.842)	1.405 (0.863-2.285)	1.350 (0.935-1.948)	1.039 (0.688-1.571)
2013 vs 2012	0.710 (0.455-1.108)	0.622 (0.394-0.980)	0.738 (0.487-1.118)	0.799 (0.545-1.171)
2014 vs 2013	0.643(0.426-0.970)	0.877(0.533-1.442)	0.492(0.316-0.765)	0.972(0.653-1.447)
2015 vs 2014	0.807 (0.540-1.206)	0.767 (0.446-1.318)	1.451 (0.904-2.330)	0.630 (0.416-0.955)
2016 vs 2015	1.326 (0.879-2.000)	0.947 (0.541-1.658)	0.931 (0.578-1.501)	1.422 (0.932 -2.170)
2017 vs 2016	0.949 (0.624-1.442)	1.533 (0.853-2.755)	0.931 (0.584-1.482)	1.124 (0.739-1.712)

➢ Index of diversity: Weighted Entropy

The weighted entropy is a diversity index that reflects how many different patterns of resistance are present in a dataset, and simultaneously take into account how evenly the observed resistance patterns are distributed. The weighted entropy takes a value (loser to 1 if the isolates are resistant to a higher number of antimicrobials. As shown in **table 9**, the value of the index globally decreased over time for all species from 2012 to 2016 but increase in 2017 in all species and especially in beef cattle. The index is globally lower for pigs compared with other species.

Table 9. Weighted Entropy by species category and by years.

Years	Veal calves	Beef cattle	Chickens	Pigs
2011	0.68	0.52	0.64	0.48
2012	0.7	0.63	0.79	0.48
2013	0.63	0.55	0.62	0.4
2014	0.54	0.59	0.59	0.32
2015	0.54	0.48	0.57	0.33
2016	0.50	0.41	0.58	0.36
2017	0.54	0.67	0.61	0.43

Discussion

Prevalences

Eleven substances were tested phenotypically from 2011 to 2017 and 3 from 2014 to 2017 but confirmation of the resistance was not performed. The three antimicrobials tested from 2014 are not used in veterinary medicine and prevalence of resistance was low (max 5%).

Discussion will focus now on the other eleven antimicrobials common for the seven years. The prevalence of resistance increased for 7/11 antimicrobial substances tested. in 2017 compared to 2016 in veal calves, 8/11 in beef cattle, 5/11 in chickens and 9/11 in pigs.
The prevalence of resistance for SMX and COL decreased or is still 0% when comparing 2017 to 2016 in all animal categories. The highest increases are seen for TMP (+13.79%) in veal calves, for FOT and TAZ (critical antimicrobials)(+ 9.95%, + 8.63% respectively) in chickens, FOT and TAZ (+ 8.42%, +7.86% respectively) in pigs, for CIP (critical antimicrobial)(+6.82%), TMP (+5.57%) and FOT (+5.53%) in beef cattle. The prevalence of resistance to the critical antimicrobials (CIP, FOT and TAZ) increased in every species in 2017 but at different degrees. There is globally a high level of resistance to AMP, SMX, TET and TMP in all animal species, but to a lesser extent in beef cattle. The common patterns of resistance to AMP, SMX, TMP and TET and combinations thereof often feature as a component of multi-resistance patterns, and are probably related to the presence of class 1 or class 2 integrons, which generally carry genes conferring resistance to these antimicrobials (Marchant et al., 2013). Although other risk factors have been described, antimicrobial use is recognized as the main selector for antimicrobial resistance and a correlation with resistance was pointed out in Belgium (Callens et al., 2017). In Belgium, antimicrobial sales data for use in animals are being collected on an annual basis since 2009 (BelVet-SAC, 2016). In 2016, a decrease of 20,0% in the sales of antimicrobials has been observed since 2011 and this reduction continued in 2017 (AMCRA, personal communication).

Trend analysis

GEE and NL mixed multivariate models present the lowest AIC but the other models globally gave similar results.

Linear multivariate model (GEE)

Considering the data from seven consecutive years (2011 to 2017), the probability of *E. coli* to be antimicrobial resistant is overall significantly decreasing in Belgian production animals but with a lesser extend to pigs. However, when comparing to the report from last year (2011 to 2016), there are more antimicrobials for which GEE results are non-significant in 2017 in veal calves (1 (FOT) versus 3 (GEN, FOT, TAZ)) and in beef cattle (2 (COL, GEN) versus 4 (CHL, FOT, TAZ, GEN)). However, in these species, these substances present resistance prevalences globally low (<10%) to very low (<5%). In the pigs, the situation is similar to last year (6 versus 6: AMP,CHL TAZ, FOT, GEN, COL) but in chicken, there is an improvement (3 (CIP, AMP, GEN) versus 1 (GEN)).

Specific assessments

Veal calves

The levels of antimicrobial resistance are very high in veal calves for AMP, SMX and TET (more than 50% of isolates are resistant during the seven consecutive years). TMP which prevalence of resistance was below 50%, since 2015 showed the most important increase observed in 2017 (+13.8%). This increase is limit to be significant by NL mixed multivariate model and significant by NL logistic.
The GEE model highlighted a significant decrease in resistance, except for FOT, GEN, TAZ (non-significant but prevalences are low to extremely low). However, it cannot be affirmed by the non-linear analysis that the significant decreases observed for from 2011 to 2014-2015, depending on the substance, continued afterward, except for NAL by NL mixed multivariate model (however limit to be non-significant in 2017). *A contrario*, an increase is observed for TMP which is significant considering the NL logistic regression and limit to be significant

considering non-linear mixed multivariate model (lower 95%CI limit= 0.99). Attention should be given to resistance in calves because we observe in 2017 OR>1 for 9/11 substances.

Beef cattle
In beef cattle, resistance prevalence is globally lower than in other species. For AMP, CIP, FOT, NAL, TAZ, TMP a non-significant increase (OR>1) is highlighted by NL mixed multivariate in 2017 (also by logistic procedure but OR of TMP is 0.999 for this substance). We should pay attention to these substances for which prevalence increased in 2017.

Chickens
Chickens present a high level of resistance to certain substances (e.i. AMP, SMX, CIP are >50% resistance during the 7 years). COL prevalence of resistance is 0% since 2014.
Based on the GEE, the probability to be resistant for substances with high levels of resistance statistically significantly decreased over time. An increasing trend was previously detected by NL models for CIP in 2012 but afterwards, a constant decrease of resistance has been observed, significant since the last years. Globally, whatever the NL model used, there is a decreasing trend in resistance in CHL, CIP (significant), COL (significant), NAL (significant).
The high resistance to quinolones in chickens is especially worrisome because of a higher resistance percentage for ciprofloxacin compared to NAL, suggesting the presence of plasmid mediated quinolone resistance (Strahilevitz et al., 2009).
There are still more than 88% of *E. coli* chicken strains are resistant to at least one of the antimicrobials in the panel.

Pigs
The prevalence of resistance for TET and SMX was above 40% during the seven consecutive years. AMP prevalence has constantly increased since 2015 and is in 2017 and for the first time, the antimicrobial with the highest prevalence in pigs. Based on the results of the GEE, a significant decrease of resistance over time was observed only for SMX, TMP, TET, CIP and NAL. A significant increase in resistance is observed at least since 2017 by both NL models for AMP, CIP, FOT and TAZ.

Multi-resistance

The proportion of **full sensitive and non-multiresistant** strains seems stable over time.
The proportion of **multi-resistant strains** (= strains resistant to at least three antimicrobials) was very high for broiler chickens (>62%) and high for veal calves (>50%) during the seven consecutive years. After four consecutive years of decrease, multi-resistance increased in beef cattle in 2017 (+ 6.59%). 25.95%, 72.50%, 11.32%, 27.12%, of, respectively, calves, cattle, chicken and pig isolates, were fully susceptible (=no resistance) in 2017 to all tested antimicrobials.
From the linear and non-linear models and for all species, significant decreases in multi-resistance were observed from 2011 onwards but progressively faded out across the last few years.

ANNEX

List of antimicrobials tested in this report and Epidemiological cut-off values (ECOFF)

Resistant strain if MIC value of the isolate > Cut-off

Symbol	Antimicrobial	Cut-off value (mg/ml)
AMP	Ampicillin	8
AZI	Azithromycin	16
CHL	Chloramphenicol	16
CIP	Ciprofloxacin	0,064
COL	Colistin	2
FOT	Cefotaxime	0,25
GEN	Gentamicin	2
MER	Meropenem	0.125
NAL	Nalidixic acid	16
SMX	Sulphonamide	64
TAZ	Ceftazidime	0,5
TET	Tetracycline	8
TGC	Tigecyclin	1
TMP	Trimethoprim	2

Outputs of the univariate logistic regression model (2011-2017)

The LOGISTIC Procedure

SPECIES= veal calves

Wald Confidence Interval for Odds Ratios			
Label	**Estimate**	**95% Confidence Limits**	
year at substance=AMP	0.883	0.825	0.945
year at substance=CHL	0.869	0.809	0.934
year at substance=CIP	0.827	0.767	0.892
year at substance=COL	0.682	0.560	0.831
year at substance=FOT	0.886	0.751	1.046
year at substance=GEN	0.872	0.763	0.997
year at substance=NAL	0.768	0.709	0.833
year at substance=SMX	0.852	0.795	0.913
year at substance=TAZ	0.856	0.727	1.006
year at substance=TET	0.890	0.828	0.957

Wald Confidence Interval for Odds Ratios			
Label	Estimate	95% Confidence Limits	
year at substance=TMP	0.847	0.792	0.905

The LOGISTIC Procedure

SPECIES= beef cattle

Wald Confidence Interval for Odds Ratios			
Label	Estimate	95% Confidence Limits	
year at substance=AMP	0.865	0.802	0.933
year at substance=CHL	0.932	0.854	1.017
year at substance=CIP	0.880	0.793	0.977
year at substance=COL	0.708	0.504	0.996
year at substance=FOT	0.926	0.792	1.084
year at substance=GEN	1.047	0.910	1.206
year at substance=NAL	0.828	0.742	0.925
year at substance=SMX	0.892	0.834	0.954
year at substance=TAZ	0.872	0.735	1.034
year at substance=TET	0.910	0.845	0.981
year at substance=TMP	0.865	0.798	0.938

The LOGISTIC Procedure

SPECIES= chickens

Wald Confidence Interval for Odds Ratios1.006			
Label	Estimate	95% Confidence Limits0.981	
year at substance=AMP	0.935	0.880	0.993
year at substance=CHL	0.928	0.879	0.980
year at substance=CIP	0.919	0.873	0.968
year at substance=COL	0.694	0.522	0.921
year at substance=FOT	0.876	0.817	0.939
year at substance=GEN	1.057	0.953	1.173

Wald Confidence Interval for Odds Ratios1.006			
Label	Estimate	95% Confidence Limits0.981	
year at substance=NAL	0.876	0.833	0.921
year at substance=SMX	0.897	0.851	0.945
year at substance=TAZ	0.873	0.812	0.938
year at substance=TET	0.872	0.830	0.917
year at substance=TMP	0.905	0.862	0.951

The LOGISTIC Procedure

SPECIES= pigs

Wald Confidence Interval for Odds Ratios			
Label	Estimate	95% Confidence Limits	
year at substance=AMP	0.997	0.943	1.055
year at substance=CHL	0.960	0.901	1.024
year at substance=CIP	0.846	0.758	0.944
year at substance=COL	0.971	0.741	1.273
year at substance=FOT	1.249	1.060	1.472
year at substance=GEN	0.841	0.674	1.049
year at substance=NAL	0.700	0.606	0.807
year at substance=SMX	0.918	0.868	0.971
year at substance=TAZ	1.173	1.003	1.373
year at substance=TET	0.911	0.861	0.964
year at substance=TMP	0.944	0.892	0.998

Outputs of the univariate logistic regression model, year by year

The LOGISTIC Procedure

SPECIES= veal calves

Wald Confidence Interval for Odds Ratios AMP			
Label	**Estimate**	**95% Confidence Limits**	
year0 at year0=0	0.639	0.491	0.831
year0 at year0=1	0.714	0.595	0.855
year0 at year0=2	0.797	0.717	0.887
year0 at year0=3	0.891	0.831	0.955
year0 at year0=4	0.996	0.887	1.117
year0 at year0=5	1.112	0.919	1.347
year0 at year0=6	1.243	0.946	1.634

Wald Confidence Interval for Odds Ratios CHL			
Label	**Estimate**	**95% Confidence Limits**	
year0 at year0=0	0.616	0.478	0.795
year0 at year0=1	0.697	0.586	0.828
year0 at year0=2	0.787	0.713	0.870
year0 at year0=3	0.890	0.829	0.956
year0 at year0=4	1.006	0.889	1.138
year0 at year0=5	1.137	0.930	1.390
year0 at year0=6	1.285	0.968	1.707

Wald Confidence Interval for Odds Ratios CIP			
Label	**Estimate**	**95% Confidence Limits**	
year0 at year0=0	0.613	0.472	0.797
year0 at year0=1	0.683	0.572	0.816
year0 at year0=2	0.761	0.688	0.843
year0 at year0=3	0.849	0.786	0.916
year0 at year0=4	0.946	0.828	1.080
year0 at year0=5	1.054	0.851	1.306
year0 at year0=6	1.175	0.869	1.587

Wald Confidence Interval for Odds Ratios COL			
Label	Estimate	95% Confidence Limits	
year0 at year0=0	0.628	0.357	1.103
year0 at year0=1	0.651	0.453	0.936
year0 at year0=2	0.675	0.547	0.833
year0 at year0=3	0.700	0.555	0.885
year0 at year0=4	0.726	0.485	1.087
year0 at year0=5	0.753	0.410	1.383
year0 at year0=6	0.781	0.344	1.775

Wald Confidence Interval for Odds Ratios FOT			
Label	Estimate	95% Confidence Limits	
year0 at year0=0	0.503	0.300	0.843
year0 at year0=1	0.619	0.438	0.875
year0 at year0=2	0.763	0.627	0.928
year0 at year0=3	0.940	0.805	1.097
year0 at year0=4	1.158	0.878	1.526
year0 at year0=5	1.426	0.917	2.218
year0 at year0=6	1.757	0.948	3.256

Wald Confidence Interval for Odds Ratios GEN			
Label	Estimate	95% Confidence Limits	
year0 at year0=0	0.707	0.455	1.097
year0 at year0=1	0.764	0.569	1.026
year0 at year0=2	0.826	0.697	0.977
year0 at year0=3	0.892	0.780	1.022
year0 at year0=4	0.965	0.761	1.223

Wald Confidence Interval for Odds Ratios GEN			
Label	Estimate	95% Confidence Limits	
year0 at year0=5	1.043	0.715	1.521
year0 at year0=6	1.127	0.666	1.907

Wald Confidence Interval for Odds Ratios NAL			
Label	Estimate	95% Confidence Limits	
year0 at year0=0	0.742	0.565	0.974
year0 at year0=1	0.752	0.626	0.902
year0 at year0=2	0.762	0.686	0.846
year0 at year0=3	0.772	0.708	0.841
year0 at year0=4	0.782	0.673	0.909
year0 at year0=5	0.793	0.625	1.006
year0 at year0=6	0.803	0.577	1.119

Wald Confidence Interval for Odds Ratios SMX			
Label	Estimate	95% Confidence Limits	
year0 at year0=0	0.596	0.454	0.784
year0 at year0=1	0.673	0.557	0.813
year0 at year0=2	0.760	0.680	0.850
year0 at year0=3	0.858	0.799	0.921
year0 at year0=4	0.968	0.862	1.087
year0 at year0=5	1.093	0.900	1.327
year0 at year0=6	1.233	0.933	1.629

Wald Confidence Interval for Odds Ratios TAZ			
Label	Estimate	95% Confidence Limits	
year0 at year0=0	0.482	0.293	0.794
year0 at year0=1	0.597	0.428	0.832
year0 at year0=2	0.740	0.613	0.892
year0 at year0=3	0.916	0.786	1.067
year0 at year0=4	1.135	0.864	1.490
year0 at year0=5	1.405	0.910	2.169
year0 at year0=6	1.740	0.951	3.186

Wald Confidence Interval for Odds Ratios TET			
Label	Estimate	95% Confidence Limits	
year0 at year0=0	0.732	0.553	0.968
year0 at year0=1	0.782	0.645	0.949
year0 at year0=2	0.836	0.746	0.937
year0 at year0=3	0.894	0.830	0.962
year0 at year0=4	0.956	0.846	1.079
year0 at year0=5	1.022	0.835	1.250
year0 at year0=6	1.092	0.818	1.458

Wald Confidence Interval for Odds Ratios TMP			
Label	Estimate	95% Confidence Limits	
year0 at year0=0	0.483	0.371	0.628
year0 at year0=1	0.586	0.489	0.702
year0 at year0=2	0.710	0.638	0.790
year0 at year0=3	0.861	0.804	0.922
year0 at year0=4	1.044	0.932	1.170
year0 at year0=5	1.266	1.047	1.531
year0 at year0=6	1.535	1.170	2.015

The LOGISTIC Procedure

species= beef cattle

Wald Confidence Interval for Odds Ratios AMP			
Label	**Estimate**	**95% Confidence Limits**	
year0 at year0=0	0.761	0.615	0.942
year0 at year0=1	0.804	0.701	0.922
year0 at year0=2	0.849	0.784	0.920
year0 at year0=3	0.897	0.818	0.984
year0 at year0=4	0.948	0.808	1.112
year0 at year0=5	1.001	0.789	1.271
year0 at year0=6	1.058	0.768	1.458

Wald Confidence Interval for Odds Ratios CHL			
Label	**Estimate**	**95% Confidence Limits**	
year0 at year0=0	0.935	0.726	1.205
year0 at year0=1	0.934	0.794	1.099
year0 at year0=2	0.933	0.848	1.025
year0 at year0=3	0.931	0.837	1.037
year0 at year0=4	0.930	0.774	1.119
year0 at year0=5	0.929	0.705	1.225
year0 at year0=6	0.928	0.639	1.347

Wald Confidence Interval for Odds Ratios CIP			
Label	**Estimate**	**95% Confidence Limits**	
year0 at year0=0	0.683	0.513	0.910
year0 at year0=1	0.762	0.635	0.915
year0 at year0=2	0.851	0.766	0.945

Wald Confidence Interval for Odds Ratios CIP			
Label	**Estimate**	**95% Confidence Limits**	
year0 at year0=3	0.949	0.838	1.075
year0 at year0=4	1.059	0.854	1.314
year0 at year0=5	1.182	0.856	1.631
year0 at year0=6	1.319	0.855	2.034

Wald Confidence Interval for Odds Ratios COL			
Label	**Estimate**	**95% Confidence Limits**	
year0 at year0=0	1.074	0.439	2.629
year0 at year0=1	0.864	0.512	1.455
year0 at year0=2	0.695	0.463	1.041
year0 at year0=3	0.558	0.280	1.114
year0 at year0=4	0.449	0.150	1.349
year0 at year0=5	0.361	0.078	1.680
year0 at year0=6	0.290	0.040	2.113

Wald Confidence Interval for Odds Ratios FOT			
Label	**Estimate**	**95% Confidence Limits**	
year0 at year0=0	0.649	0.422	0.997
year0 at year0=1	0.755	0.573	0.993
year0 at year0=2	0.878	0.751	1.026
year0 at year0=3	1.021	0.856	1.217
year0 at year0=4	1.187	0.871	1.618
year0 at year0=5	1.381	0.865	2.204
year0 at year0=6	1.606	0.853	3.022

Wald Confidence Interval for Odds Ratios GEN			
Label	**Estimate**	**95% Confidence Limits**	
year0 at year0=0	1.265	0.807	1.985
year0 at year0=1	1.174	0.873	1.578
year0 at year0=2	1.089	0.917	1.293
year0 at year0=3	1.010	0.853	1.195
year0 at year0=4	0.937	0.701	1.252
year0 at year0=5	0.869	0.558	1.354
year0 at year0=6	0.806	0.440	1.476

Wald Confidence Interval for Odds Ratios NAL			
Label	**Estimate**	**95% Confidence Limits**	
year0 at year0=0	0.678	0.505	0.910
year0 at year0=1	0.742	0.616	0.893
year0 at year0=2	0.811	0.728	0.905
year0 at year0=3	0.888	0.774	1.018
year0 at year0=4	0.971	0.767	1.228
year0 at year0=5	1.062	0.750	1.505
year0 at year0=6	1.162	0.729	1.851

Wald Confidence Interval for Odds Ratios SMX			
Label	**Estimate**	**95% Confidence Limits**	
year0 at year0=0	0.902	0.741	1.098
year0 at year0=1	0.898	0.791	1.019
year0 at year0=2	0.894	0.831	0.962
year0 at year0=3	0.890	0.819	0.967
year0 at year0=4	0.886	0.768	1.021
year0 at year0=5	0.881	0.711	1.092

Wald Confidence Interval for Odds Ratios SMX			
Label	Estimate	95% Confidence Limits	
year0 at year0=6	0.877	0.657	1.171

Wald Confidence Interval for Odds Ratios TAZ			
Label	Estimate	95% Confidence Limits	
year0 at year0=0	0.656	0.416	1.034
year0 at year0=1	0.744	0.557	0.992
year0 at year0=2	0.843	0.714	0.995
year0 at year0=3	0.956	0.782	1.169
year0 at year0=4	1.084	0.765	1.536
year0 at year0=5	1.229	0.730	2.069
year0 at year0=6	1.393	0.692	2.8

Wald Confidence Interval for Odds Ratios TET			
Label	Estimate	95% Confidence Limits	
year0 at year0=0	0.884	0.713	1.098
year0 at year0=1	0.895	0.779	1.029
year0 at year0=2	0.906	0.836	0.983
year0 at year0=3	0.917	0.837	1.005
year0 at year0=4	0.929	0.793	1.087
year0 at year0=5	0.940	0.742	1.191
year0 at year0=6	0.952	0.692	1.309

Wald Confidence Interval for Odds Ratios TMP			
Label	Estimate	95% Confidence Limits	
year0 at year0=0	0.790	0.629	0.993
year0 at year0=1	0.822	0.711	0.950

Wald Confidence Interval for Odds Ratios TMP			
Label	**Estimate**	**95% Confidence Limits**	
year0 at year0=2	0.855	0.785	0.930
year0 at year0=3	0.889	0.804	0.983
year0 at year0=4	0.924	0.777	1.099
year0 at year0=5	0.961	0.742	1.244
year0 at year0=6	0.999	0.707	1.413

The LOGISTIC Procedure

species=chickens

Wald Confidence Interval for Odds Ratios AMP			
Label	**Estimate**	**95% Confidence Limits**	
year0 at year0=0	0.853	0.710	1.024
year0 at year0=1	0.886	0.789	0.996
year0 at year0=2	0.922	0.862	0.985
year0 at year0=3	0.959	0.887	1.036
year0 at year0=4	0.997	0.870	1.142
year0 at year0=5	1.036	0.845	1.272
year0 at year0=6	1.078	0.818	1.420

Wald Confidence Interval for Odds Ratios CHL			
Label	**Estimate**	**95% Confidence Limits**	
year0 at year0=0	1.077	0.921	1.260
year0 at year0=1	1.008	0.914	1.112
year0 at year0=2	0.943	0.891	1.000
year0 at year0=3	0.883	0.819	0.952
year0 at year0=4	0.827	0.726	0.941
year0 at year0=5	0.774	0.639	0.936

Wald Confidence Interval for Odds Ratios CHL			
Label	**Estimate**	**95% Confidence Limits**	
year0 at year0=6	0.724	0.561	0.934

Wald Confidence Interval for Odds Ratios CIP			
Label	**Estimate**	**95% Confidence Limits**	
year0 at year0=0	1.238	1.063	1.443
year0 at year0=1	1.090	0.990	1.200
year0 at year0=2	0.959	0.909	1.013
year0 at year0=3	0.845	0.791	0.901
year0 at year0=4	0.743	0.663	0.834
year0 at year0=5	0.654	0.550	0.778
year0 at year0=6	0.576	0.456	0.727

Wald Confidence Interval for Odds Ratios COL			
Label	**Estimate**	**95% Confidence Limits**	
year0 at year0=0	8.001	2.142	29.892
year0 at year0=1	0.387	0.143	1.045
year0 at year0=2	0.019	0.002	0.232
year0 at year0=3	<0.001	<0.001	0.061
year0 at year0=4	<0.001	<0.001	0.017
year0 at year0=5	<0.001	<0.001	0.005
year0 at year0=6	<0.001	<0.001	0.001

Wald Confidence Interval for Odds Ratios FOT			
Label	**Estimate**	**95% Confidence Limits**	
year0 at year0=0	0.653	0.540	0.791
year0 at year0=1	0.746	0.663	0.841

Wald Confidence Interval for Odds Ratios FOT			
Label	Estimate	95% Confidence Limits	
year0 at year0=2	0.852	0.796	0.912
year0 at year0=3	0.974	0.891	1.064
year0 at year0=4	1.112	0.952	1.298
year0 at year0=5	1.270	1.009	1.599
year0 at year0=6	1.450	1.066	1.974

Wald Confidence Interval for Odds Ratios GEN			
Label	Estimate	95% Confidence Limits	
year0 at year0=0	1.117	0.814	1.533
year0 at year0=1	1.092	0.893	1.336
year0 at year0=2	1.067	0.951	1.197
year0 at year0=3	1.043	0.913	1.190
year0 at year0=4	1.019	0.808	1.285
year0 at year0=5	0.996	0.702	1.412
year0 at year0=6	0.973	0.607	1.559

Wald Confidence Interval for Odds Ratios NAL			
Label	Estimate	95% Confidence Limits	
year0 at year0=0	1.105	0.950	1.284
year0 at year0=1	1.000	0.910	1.100
year0 at year0=2	0.906	0.858	0.956
year0 at year0=3	0.820	0.769	0.875
year0 at year0=4	0.743	0.663	0.832
year0 at year0=5	0.673	0.567	0.798
year0 at year0=6	0.609	0.484	0.766

Wald Confidence Interval for Odds Ratios SMX			
Label	Estimate	95% Confidence Limits	
year0 at year0=0	0.851	0.726	0.998
year0 at year0=1	0.870	0.786	0.963
year0 at year0=2	0.890	0.840	0.942
year0 at year0=3	0.910	0.851	0.973
year0 at year0=4	0.930	0.827	1.046
year0 at year0=5	0.951	0.797	1.135
year0 at year0=6	0.973	0.767	1.234

Wald Confidence Interval for Odds Ratios TAZ			
Label	Estimate	95% Confidence Limits	
year0 at year0=0	0.672	0.551	0.819
year0 at year0=1	0.757	0.669	0.857
year0 at year0=2	0.853	0.795	0.916
year0 at year0=3	0.961	0.875	1.055
year0 at year0=4	1.083	0.921	1.274
year0 at year0=5	1.220	0.959	1.553
year0 at year0=6	1.375	0.996	1.897

Wald Confidence Interval for Odds Ratios TET			
Label	Estimate	95% Confidence Limits	
year0 at year0=0	0.809	0.699	0.937
year0 at year0=1	0.836	0.762	0.917
year0 at year0=2	0.863	0.819	0.910
year0 at year0=3	0.892	0.837	0.950
year0 at year0=4	0.921	0.825	1.029
year0 at year0=5	0.951	0.806	1.123
year0 at year0=6	0.983	0.786	1.229

Wald Confidence Interval for Odds Ratios TMP			
Label	**Estimate**	**95% Confidence Limits**	
year0 at year0=0	0.908	0.785	1.051
year0 at year0=1	0.907	0.827	0.995
year0 at year0=2	0.906	0.859	0.955
year0 at year0=3	0.904	0.849	0.964
year0 at year0=4	0.903	0.808	1.009
year0 at year0=5	0.902	0.764	1.065
year0 at year0=6	0.901	0.721	1.126

The LOGISTIC Procedure

species=pigs

Wald Confidence Interval for Odds Ratios AMP			
Label	**Estimate**	**95% Confidence Limits**	
year0 at year0=0	0.786	0.661	0.936
year0 at year0=1	0.864	0.770	0.968
year0 at year0=2	0.948	0.888	1.013
year0 at year0=3	1.041	0.977	1.109
year0 at year0=4	1.143	1.024	1.277
year0 at year0=5	1.255	1.060	1.487
year0 at year0=6	1.379	1.093	1.738

Wald Confidence Interval for Odds Ratios CHL			
Label	**Estimate**	**95% Confidence Limits**	
year0 at year0=0	0.906	0.745	1.101
year0 at year0=1	0.927	0.816	1.054
year0 at year0=2	0.949	0.882	1.022
year0 at year0=3	0.972	0.903	1.045

Wald Confidence Interval for Odds Ratios CHL			
Label	**Estimate**	**95% Confidence Limits**	
year0 at year0=4	0.995	0.876	1.129
year0 at year0=5	1.018	0.839	1.237
year0 at year0=6	1.042	0.800	1.359

Wald Confidence Interval for Odds Ratios CIP			
Label	**Estimate**	**95% Confidence Limits**	
year0 at year0=0	0.441	0.325	0.598
year0 at year0=1	0.580	0.477	0.704
year0 at year0=2	0.763	0.685	0.849
year0 at year0=3	1.004	0.893	1.127
year0 at year0=4	1.321	1.070	1.630
year0 at year0=5	1.738	1.260	2.397
year0 at year0=6	2.286	1.476	3.541

Wald Confidence Interval for Odds Ratios COL			
Label	**Estimate**	**95% Confidence Limits**	
year0 at year0=0	0.945	0.421	2.122
year0 at year0=1	0.956	0.563	1.622
year0 at year0=2	0.967	0.713	1.311
year0 at year0=3	0.978	0.720	1.327
year0 at year0=4	0.989	0.582	1.682
year0 at year0=5	1.001	0.445	2.252
year0 at year0=6	1.012	0.335	3.057

Wald Confidence Interval for Odds Ratios FOT			
Label	Estimate	95% Confidence Limits	
year0 at year0=0	0.323	0.190	0.547
year0 at year0=1	0.531	0.378	0.746
year0 at year0=2	0.874	0.735	1.039
year0 at year0=3	1.439	1.242	1.667
year0 at year0=4	2.368	1.752	3.200
year0 at year0=5	3.898	2.395	6.344
year0 at year0=6	6.417	3.254	12.654

Wald Confidence Interval for Odds Ratios GEN			
Label	Estimate	95% Confidence Limits	
year0 at year0=0	0.740	0.406	1.350
year0 at year0=1	0.784	0.534	1.149
year0 at year0=2	0.830	0.664	1.037
year0 at year0=3	0.879	0.673	1.148
year0 at year0=4	0.931	0.588	1.473
year0 at year0=5	0.985	0.497	1.954
year0 at year0=6	1.044	0.416	2.615

Wald Confidence Interval for Odds Ratios NAL			
Label	Estimate	95% Confidence Limits	
year0 at year0=0	0.438	0.308	0.622
year0 at year0=1	0.546	0.438	0.680
year0 at year0=2	0.680	0.598	0.772
year0 at year0=3	0.847	0.717	1.000
year0 at year0=4	1.055	0.793	1.404
year0 at year0=5	1.315	0.861	2.007

sciensano

Wald Confidence Interval for Odds Ratios NAL			
Label	**Estimate**	**95% Confidence Limits**	
year0 at year0=6	1.638	0.931	2.882

Wald Confidence Interval for Odds Ratios SMX			
Label	**Estimate**	**95% Confidence Limits**	
year0 at year0=0	0.848	0.711	1.010
year0 at year0=1	0.875	0.779	0.981
year0 at year0=2	0.902	0.844	0.964
year0 at year0=3	0.931	0.874	0.992
year0 at year0=4	0.961	0.861	1.072
year0 at year0=5	0.991	0.837	1.174
year0 at year0=6	1.023	0.811	1.290

Wald Confidence Interval for Odds Ratios TAZ			
Label	**Estimate**	**95% Confidence Limits**	
year0 at year0=0	0.357	0.217	0.585
year0 at year0=1	0.557	0.405	0.766
year0 at year0=2	0.870	0.737	1.026
year0 at year0=3	1.358	1.176	1.568
year0 at year0=4	2.121	1.592	2.825
year0 at year0=5	3.312	2.090	5.248
year0 at year0=6	5.171	2.726	9.810

Wald Confidence Interval for Odds Ratios TET			
Label	**Estimate**	**95% Confidence Limits**	
year0 at year0=0	0.768	0.644	0.915
year0 at year0=1	0.821	0.732	0.922

Wald Confidence Interval for Odds Ratios TET			
Label	Estimate	95% Confidence Limits	
year0 at year0=2	0.879	0.822	0.939
year0 at year0=3	0.940	0.882	1.001
year0 at year0=4	1.005	0.901	1.122
year0 at year0=5	1.075	0.908	1.274
year0 at year0=6	1.150	0.912	1.451

Wald Confidence Interval for Odds Ratios TMP			
Label	Estimate	95% Confidence Limits	
year0 at year0=0	0.898	0.755	1.068
year0 at year0=1	0.916	0.817	1.026
year0 at year0=2	0.934	0.874	0.997
year0 at year0=3	0.952	0.894	1.015
year0 at year0=4	0.971	0.870	1.084
year0 at year0=5	0.990	0.836	1.173
year0 at year0=6	1.010	0.801	1.273

ANNEX 2: GEE linear model with multiple comparisons corrections (p-values)

CALVES

Test	probz	Bonferroni	Linear Stepup
AMP	0.0006	0.0065	0.0009
CHL	0.0005	0.0052	0.0009
CIP	<.0001	0.0002	<.0001
COL	0.0002	0.0018	0.0004
FOT	0.2175	1.0000	0.2175
GEN	0.0673	0.7407	0.0823

Test	probz	Bonferroni	Linear Stepup
NAL	<.0001	<.0001	<.0001
SMX	<.0001	0.0001	<.0001
TAZ	0.1003	1.0000	0.1104
TET	0.0028	0.0304	0.0038
TMP	<.0001	<.0001	<.0001

CATTLE

Test	probz	Bonferroni	Linear Stepup
AMP	0.0002	0.0018	0.0017
CHL	0.0789	0.8681	0.1085
CIP	0.0220	0.2419	0.0346
COL	0.0107	0.1180	0.0197
FOT	0.3384	1.0000	0.3723
GEN	0.5961	1.0000	0.5961
NAL	0.0012	0.0133	0.0033
SMX	0.0005	0.0052	0.0017
TAZ	0.1317	1.0000	0.1610
TET	0.0095	0.1050	0.0197
TMP	0.0003	0.0035	0.0017

CHICKEN

Test	probz	Bonferroni	Linear Stepup
AMP	0.0237	0.2612	0.0261
CHL	0.0065	0.0718	0.0080
CIP	0.0021	0.0226	0.0028
COL	<.0001	<.0001	<.0001
FOT	0.0005	0.0056	0.0009
GEN	0.2280	1.0000	0.2280
NAL	<.0001	<.0001	<.0001
SMX	<.0001	0.0006	0.0001
TAZ	0.0007	0.0073	0.0010
TET	<.0001	<.0001	<.0001
TMP	<.0001	0.0010	0.0002

PIG

Test	probz	Bonferroni	Linear Stepup
AMP	0.8384	1.0000	0.9222
CHL	0.1729	1.0000	0.2378
CIP	0.0204	0.2242	0.0561
COL	0.9444	1.0000	0.9444
FOT	0.0592	0.6517	0.1086
GEN	0.1959	1.0000	0.2394
NAL	<.0001	0.0008	0.0008
SMX	0.0019	0.0204	0.0068
TAZ	0.1198	1.0000	0.1883
TET	0.0007	0.0080	0.0040

Test	probz	Bonferroni	Linear Stepup
TMP	0.0295	0.3240	0.0648

Results of the univariate (logistic regression) and multivariate (GEE) analysis are summarized hereafter in a table using simple symbols in order to get an overall picture of the situation over the seven consecutive years and to easily make comparisons between animal categories. All indicated trends (↑, ↓) were statistically significant (p = 0.05) both in univariate (logistic regression) and multivariate (GEE) analysis, even after using correction methods for multiple testing (Bonferroni and Linear step-up method), unless otherwise mentioned.

	Veal Calves	Beef Cattle	Chickens	Pigs
AMP	↓	↓	↓ 1++	
CHL	↓		↓ 1	
CIP	↓	↓1	↓	↓1,2
COL	↓	↓1	↓	
FOT			↓	
GEN	3**			
NAL	↓	↓	↓	↓
SMX	↓	↓	↓++	↓
TAZ			↓	
TET	↓++	↓1	↓	↓
TMP	↓	↓	↓	↓ 1,2,3**

++ = High

prevalence (> 50%) for the 7 consecutive years
↓ = **decreasing** trend of resistance detected*
1=Trend not significant after p value adjustment with Bonferroni method
2=Trend not significant after p value adjustment with Linear method
3= Trend not significant in multivariate analysis (GEE) but significant in univariate analysis (logistic regression)

*statistically significant trend (5% significance level) detected at least once during the 7 years

**: upper limit is really close to 1 (not significant): GEN veal calves: 0.997; TMP pig: 0.998

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