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Quantitative assessment of antimicrobial resistance in livestock during the course of a nationwide antimicrobial use reduction in the Netherlands

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Objectives: To quantify associations between antimicrobial use and acquired resistance in indicator *Escherichia coli* over a period of time which involved sector-wide antimicrobial use reductions in broilers and pigs (years 2004–14), veal calves (2007–14) and dairy cattle (2005–14). Prevalence estimates of resistance were predicted for a hypothetical further decrease in antimicrobial use.

Methods: Data reported annually for the resistance surveillance programme in the Netherlands were retrieved. Two multivariate random-effects logistic models per animal sector were used to relate total and class-specific antimicrobial use (as defined daily dosages per animal per year, DDDA/Y) with the probability of *E. coli* resistance to a panel of 10 antimicrobial agents.

Results: Positive dose–response relationships (ORs) were obtained from all models. Specific resistance phenotypes were more often associated with total antimicrobial use than with class-specific use. The most robust associations were found in pigs and veal calves. Resistance to historically widely used antimicrobials (e.g. penicillins, tetracyclines) was, in relative terms, less influenced by drug use changes over time than resistance to newer or less prescribed antimicrobials (e.g. third-/fourth-generation cephalosporins, fluoroquinolones). In pigs and veal calves, prevalence estimates for the most common resistance phenotypes were projected to decline ∼5%–25% during 2014–16 if total antimicrobial use reduction reached 80%; projections for poultry and dairy cows were more modest.

Conclusions: Epidemiological evidence indicated that drug use history and co-selection of resistance are key elements for perpetuation of resistance. Data suggest that recent Dutch policies aimed at reducing total use of antimicrobials have decreased *E. coli* resistance in the pig and veal calf production sectors while the impact on the dairy cattle and poultry sectors is less clear.

Introduction

The use of antibiotics is the major driver for the emergence of bacterial resistance, which can be transmitted from food-producing animals to humans.[1–8] During recent years, many efforts have been made in Europe for a more prudent veterinary use of antimicrobials, such as the EU-wide ban on the use of growth promoters in 2006, and the development of comprehensive antimicrobial use and resistance monitoring programmes.[9–13]

In the Netherlands, regulations for farmers and veterinarians have also changed considerably in recent years.[14,15] In 2007, antimicrobial sales for food animals made the country one of the highest consumers of these products among the EU member states,[13,16] and the government, animal sectors and veterinarians initiated concerted action to tackle this situation. In 2010, an ambitious policy defined mandatory targets for veterinary antimicrobial use, aiming at reductions of 50% by 2013 and 70% by 2015 compared with the index year 2009. The first target was easily achieved in 2013.[17] Additionally, from that year on, the use of third- and fourth-generation cephalosporins and fluoroquinolones was restricted by law for infections demonstrated by bacterial culture and susceptibility test results.[18] In recent years,

resistance levels in *Campylobacter* spp. and commensal *Escherichia coli* in the main livestock sectors have decreased, which is interpreted as evidence of these measures having a positive impact.[19]

The purpose of this study was to explore and quantify the association between use of antimicrobials in animals and resistance levels in commensal indicator *E. coli* over a period that saw a major reduction in antimicrobial use within the four major livestock production sectors (broilers, pigs, veal calves and dairy cattle) in the Netherlands. Moreover, we explored a potential future scenario by predicting resistance rates that would result from a further reduction in antimicrobial use.

Materials and methods

Antimicrobial use and resistance data

A more detailed description of the data used for this study can be found in the Supplementary data (available at *JAC* Online) along with additional references.[20–24] Briefly, we retrieved the annual defined daily dosages per animal per year (DDDA/Y) reported by the Netherlands Veterinary Medicines Authority (SDa) for total use and specific antimicrobial classes until 2014 per animal sector.[17] Additionally, we used the results for resistance in *E. coli* communicated annually in the *Monitoring Antimicrobial Resistance and Antibiotic Use in Animals in the Netherlands* (MARAN) reports.[19] Consistent with the MARAN reports, the terms 'resistance' or 'resistant' in this work refer to non-WT isolates defined by epidemiological cut-off values (www.eucast.org).[19] MDR refers to isolates with non-WT susceptibility to three or more antimicrobial classes.[25]

Years in which both antimicrobial use and resistance data were available were matched. Similarly, use per antimicrobial class was matched with susceptibility tests for corresponding antimicrobials. This resulted in eight used antimicrobial classes matching 10 antibiotics tested: usage of penicillins, tetracyclines, third-/fourth-generation cephalosporins, fluoroquinolones, quinolones and amphenicols matched with acquired resistance to ampicillin, tetracycline, cefotaxime, ciprofloxacin, nalidixic acid and chloramphenicol, respectively; aggregated information by the SDa on use of trimethoprim/sulphonamides was matched with both trimethoprim and sulfamethoxazole resistance, and use of aminoglycosides with resistance to both gentamicin and streptomycin.

Statistical analysis

Resistance patterns were assessed during the study period for the panel of nine antimicrobials excluding streptomycin, which was not tested every year. The same pattern evaluation was made for the period including streptomycin. Percentages of isolates resistant to each antimicrobial, among isolates exhibiting a certain number of resistance phenotypes (from 0, fully susceptible; to 9 or 10, pan-resistant to all agents), were calculated. We defined 1 − [pan-susceptibility] as the proportion of isolates resistant to at least one of the antimicrobials (i.e. 1 − proportion of isolates susceptible to all agents). Trends for annual antimicrobial use and for prevalence of resistance were plotted using smoothed lines passing through the point estimates, and changes between the years 2009 and 2014 (from the implementation of the antimicrobial policies) were described.

Logistic regression analysis for grouped data (number of resistant isolates over the total tested) was used to obtain ORs for an *E. coli* isolate to be resistant to each antimicrobial agent (or to any agent, represented by 1 – [pan-susceptibility]) per 1 U increase in total antimicrobial use (total DDDA/Y, which included also occasional use of first-/second-generation cephalosporins, combinations of antibiotics, macrolides/lincosamides, pleuromutilins and polymyxins) or 1 U increase in homologous use (i.e. DDDA/Y corresponding to the same antimicrobial class as the agent tested). Two multivariate random-effects generalized linear mixed models were fitted per animal sector. Models were adjusted for year and included a random intercept to account for the correlation between the different antimicrobial resistance phenotypes per year. The first model explored the associations between total antimicrobial use and resistance (agent-specific and 1 − [pan-susceptibility]). The second model assessed the relationships between homologous antimicrobial use and corresponding agent resistance. For the latter, only classes with DDDA/Y > 0.5 in all years were modelled to obtain model convergence and reliable estimates. A categorical explanatory variable with the resistance types (agent-specific and 1 − [pan-susceptibility]) was included and its interaction with DDDA/Y was used to separate the different model outcomes. In veal calves, an extra variable was included in the model to adjust the OR estimates for the two different sampling frames used in this sector (i.e. until 2011 in farms and from 2012 at slaughterhouses). Model assumptions were checked with diagnostic plots. ORs and 95% CIs for the different associations were plotted. Finally, predicted *E. coli* resistance prevalence, related to a hypothetical total antimicrobial use reduction of 80% by 2016 from the index year 2009, were made per animal sector. Only the models with total antimicrobial use as a determinant were used to make the predictions. All models were fitted with PROC GLIMMIX in SAS software v. 9.2 (SAS Institute Inc., Cary, NC, USA). Sigma Plot software v. 12.5 (Systat Software Inc., San Jose, CA, USA) was used to create the graphs.

In a sensitivity analysis, we also explored the effect of a 1 year lag of antimicrobial use on resistance with the same models (e.g. relating antibiotic use in year 2013 with the resistance prevalence in 2014). These results were generally similar and are not presented.

Results

Resistance patterns

The percentages of antimicrobial resistance to each of the nine antimicrobials tested during 2004–14 (Tables S1–S4) did not fundamentally differ from those including streptomycin (i.e. 10 agents) in the period 2007–13 (Table 1). Susceptibility to all antimicrobials was highest among isolates from dairy cattle (93%) while it was relatively low in veal calves and slaughter pigs (38% and 22%, respectively), and very low in broilers (12%) (Table 1). The highest level of multidrug resistance was observed in broilers (75% of isolates), followed by pigs (55%), veal calves (45%) and dairy cattle (4%) (Table 1). Patterns of resistance visualized by shaded cells were comparable between animals; resistance phenotypes to ampicillin, tetracycline, sulfamethoxazole, trimethoprim and streptomycin dominated among most of the multiresistant isolates, except for broilers, which showed an additional dominance of ciprofloxacin and nalidixic acid resistance (Table 1).

A stratified analysis per period of time in all animal sectors (Tables S1 – S4) showed that susceptibility to all antimicrobials was higher in the last 4 years of the study when compared with the previous periods, and multidrug resistance was reduced. Nonetheless, resistance patterns did not fundamentally change over time; resistance to the most commonly used antimicrobials (Figure 1) dominated in all linked phenotypes and cefotaxime resistance was related to isolates with the highest number of resistance phenotypes (Table 1).

Long-term trends and changes in antimicrobial use and prevalence of resistance

Trends in antimicrobial use and resistance by animal sector over the study period are displayed in Figure 1. In broilers and slaughter

Table 1. Antimicrobial resistance patterns for all *E. coli* isolates obtained from the Dutch antimicrobial resistance monitoring (MARAN) in broilers, slaughter pigs, veal calves and dairy cows during the period 2007–13[a]

Animal species	No. of resistance phenotypes[b]	No. of isolates[c]	Percentage of the total isolates	Antimicrobial agent resistance (%)[d]									
				AMP	TET	SMX	TMP	CIP	NAL	CHL	CTX	STR	GEN
Broilers	fully susceptible	227	12	0	0	0	0	0	0	0	0	0	0
	resistant to 1	98	5	37	21	8	1	1	0	0	0	29	3
	resistant to 2	151	8	32	19	18	3	48	49	1	3	24	4
	resistant to 3	160	8	44	24	38	24	49	49	5	4	62	3
	resistant to 4	230	12	82	48	67	50	39	39	7	10	53	6
	resistant to 5	245	13	82	67	87	74	40	41	13	12	76	8
	resistant to 6	259	13	84	67	95	80	80	80	22	11	72	8
	resistant to 7	263	14	94	86	98	92	98	98	18	15	90	13
	resistant to 8	221	11	99	96	100	91	99	100	80	25	99	11
	resistant to 9	71	4	100	94	100	99	100	100	90	48	100	69
	pan-resistant	9	0	100	100	100	100	100	100	100	100	100	100
	total isolates	1934	100	67	54	66	55	57	57	21	12	62	10
Slaughter pigs	fully susceptible	406	22	0	0	0	0	0	0	0	0	0	1
	resistant to 1	216	12	4	62	1	2	0	0	1	0	28	2
	resistant to 2	218	12	11	65	32	19	0	0	3	0	66	3
	resistant to 3	247	14	22	84	72	45	1	0	5	0	69	2
	resistant to 4	269	15	42	77	93	86	0	0	14	3	80	4
	resistant to 5	344	19	86	98	100	98	1	1	17	1	95	2
	resistant to 6	88	5	95	99	100	99	8	9	80	6	98	7
	resistant to 7	14	1	86	100	100	86	86	86	43	21	93	0
	resistant to 8	12	1	100	100	100	100	100	100	67	33	92	8
	resistant to 9	0	0	0	0	0	0	0	0	0	0	0	0
	pan-resistant	0	0	0	0	0	0	0	0	0	0	0	0
	total isolates	1814	100	33	63	53	46	2	2	11	2	57	2
Veal calves	fully susceptible	510	38	0	0	0	0	0	0	0	0	0	1
	resistant to 1	158	12	2	94	1	0	0	0	0	0	3	1
	resistant to 2	69	5	35	90	9	13	3	3	3	1	39	4
	resistant to 3	81	6	31	90	58	25	11	11	6	0	68	0
	resistant to 4	93	7	59	89	88	60	5	5	14	1	76	1
	resistant to 5	181	14	91	99	97	77	4	4	33	2	91	2
	resistant to 6	108	8	67	99	99	94	33	34	69	2	94	9
	resistant to 7	50	4	86	98	100	86	86	78	56	2	88	20
	resistant to 8	45	3	91	98	98	98	96	93	84	11	100	31
	resistant to 9	40	3	100	100	100	100	100	100	98	5	100	98
	pan-resistant	4	0	100	100	100	100	100	100	100	100	100	100
	total isolates	1339	100	35	59	42	34	14	14	20	1	41	6
Dairy cattle	fully susceptible	1320	93	0	0	0	0	0	0	0	0	0	1
	resistant to 1	33	2	9	52	3	0	0	3	3	0	12	18
	resistant to 2	6	0	33	67	17	0	17	17	17	0	33	0
	resistant to 3	23	2	43	78	61	13	0	0	9	0	87	9
	resistant to 4	10	1	70	90	100	50	0	0	0	10	80	0
	resistant to 5	12	1	83	100	100	83	8	8	25	0	92	0

Continued

Table 1. *Continued*

Animal species	No. of resistance phenotypes[b]	No. of isolates[c]	Percentage of the total isolates	Antimicrobial agent resistance (%)[d]									
				AMP	TET	SMX	TMP	CIP	NAL	CHL	CTX	STR	GEN
	resistant to 6	7	0	57	100	100	71	29	29	43	29	100	43
	resistant to 7	2	0	100	100	100	100	100	100	0	0	100	0
	resistant to 8	5	0	100	80	100	80	100	100	80	40	100	20
	resistant to 9	4	0	100	100	100	100	100	100	100	25	100	75
	pan-resistant	1	0	100	100	100	100	100	100	100	100	100	100
	total isolates	1423	100	3	5	4	2	1	1	1	0	4	1

AMP, ampicillin; TET, tetracycline; SMX, sulfamethoxazole; TMP, trimethoprim; CIP, ciprofloxacin; NAL, nalidixic acid; CHL, chloramphenicol; CTX, cefotaxime; STR, streptomycin; GEN, gentamicin.
[a]The period 2007–13 included streptomycin in the panel of agents for antimicrobial susceptibility testing. Resistance patterns during the whole study period 2004–14 (excluding streptomycin) are presented in Tables S1–S4.
[b]Number of resistance phenotypes (0–10) to the 10 antimicrobial agents tested.
[c]Number of isolates collected between 2007 and 2013 and resistant to 0–10 antimicrobial agents.
[d]Percentage of resistance to each of the 10 antimicrobials by the number of resistance phenotypes they exhibit (from 0 to 10). Cells are gradually shaded in grey according to percentage (i.e. the larger percentage, the darker the cell).

pigs, there was a period of increasing antimicrobial use until 2009, followed by a period of marked decrease until 2014; prevalence of acquired resistance moderately paralleled the trends in antibiotic use. In veal calves, antimicrobial use and resistance decreased until 2014 but an abrupt difference in resistance was evident before and after 2012, probably because of the change in sampling strategy. The use of antimicrobials in dairy cattle was stable at a low level while the low-resistance prevalence sharply increased in 2009. Animal sectors had different regimens of drug prescription, but, in general, tetracyclines, penicillins and trimethoprim/sulphonamides were the most frequently used drugs in all sectors, with a substantial contribution of quinolones in broiler production (Figure 1).

Changes in antimicrobial use and resistance prevalence from the index year 2009 to 2014 are shown in Table 2. In broilers and slaughter pigs, relative decreases in use of the most commonly administered antimicrobials were the most dramatic (from −57% to −70% in broilers for total use and specific use of tetracyclines and quinolones; and from −54% to −63% in pigs for total and specific use of tetracyclines and trimethoprim/sulphonamides). However, in broilers, the relative decrease in prevalence of resistance to these drugs was more limited (from −8% to −31%) than in pigs (from −22% to −43%). In the veal calf sector, a slightly more moderate decrease in use of the most common antimicrobials (from −40% to −44%) was paired with the most dramatic drop in prevalence of common resistance phenotypes (from −25% to −46%), potentially biased by the change in sampling strategy. In dairy cattle, antimicrobial use and resistance levels remained very low except for the unexplained sharp increase in 2009 that made the interpretation of relative changes difficult and unreliable. In all sectors, as a result of the implemented restriction policies, use of third-/fourth-generation cephalosporins and fluoroquinolones was almost completely absent in 2014, and this was accompanied by noticeable changes in resistance when comparing 2009 with 2014 levels (−84% reduction of cefotaxime resistance and −19% reduction of ciprofloxacin resistance in broilers, −86% and −100% in pigs, −41% and −64% in veal calves, and −75% and −100% in dairy cattle) (Table 2).

Associations between antimicrobial use and resistance

Dose–response antimicrobial use–resistance associations expressed as ORs and 95% CIs are shown in Figure 2. The numerical outcomes of the models are presented in Table 3. As a rule, the probability of *E. coli* resistant isolates was higher with increased use of antimicrobials (i.e. ORs > 1). The different resistance phenotypes were more significantly associated (i.e. narrower CIs and ORs >1) with total antimicrobial use (Figure 2a) than with homologous use (Figure 2b).

In broilers, the probability of an isolate being resistant to any of the antibiotics was from 1% to 5% higher per unit increase in total DDDA/Y; these associations were statistically significant or borderline significant (i.e. CIs for ORs ≥1), except for 1 − [pan-susceptibility], ampicillin and streptomycin resistance. Increased use in homologous antimicrobial classes was related to higher probabilities (e.g. fluoroquinolone use–ciprofloxacin resistance, OR = 1.42). However, these homologous associations were often less significant (Figure 2a and b, Table 3).

In pigs, total antimicrobial use was positively related to resistance with higher probabilities than in broilers. One unit increase in DDDA/Y was associated with a ∼8% increased odds of total, tetracycline, ampicillin, trimethoprim and sulfamethoxazole resistance, and with a ∼30%–40% increased probability of ciprofloxacin and nalidixic acid resistance. In models with homologous use, ORs were never statistically significant (Figure 2a and b, Table 3).

In veal calves, the ORs for the relationships between total antimicrobial use and resistance were similar and statistically significant for most of the resistance phenotypes (ORs between 1.07 and 1.17). Statistical significance was lost in models accounting for the change in sampling strategy. Estimates from the models

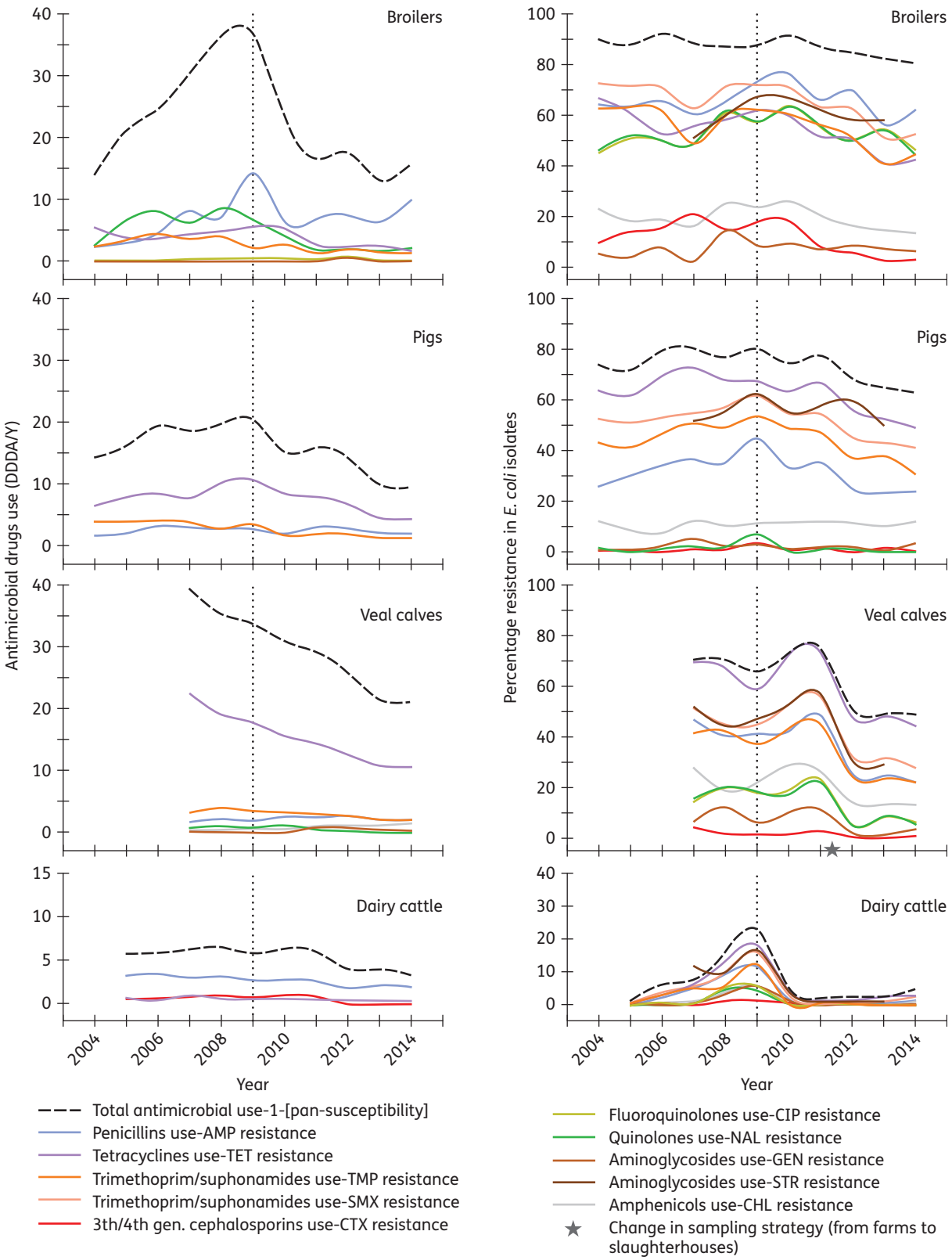

Figure 1. Antimicrobial drug use (as defined daily dosages per animal per year; DDDA/Y) (left) and percentages of resistant *E. coli* isolates (right) for broilers, pigs, veal calves and dairy cows before and after the implementation of a policy to reduce antimicrobial use (index year 2009 represented as the vertical dotted line) in the Netherlands 2004–14. Antimicrobial classes with use below 0.5 DDDA/Y in all years are not shown. 1 − [pan-susceptibility] refers to isolates resistant to at least one of the agents of the susceptibility testing panel (i.e. 1 − proportion of fully susceptible isolates). AMP, ampicillin; TET, tetracycline; SMX, sulfamethoxazole; TMP, trimethoprim; CIP, ciprofloxacin; NAL, nalidixic acid; CHL, chloramphenical, CTX, cefotaxime; STR, streptomycin; GEN, gentamicin.

Table 2. Changes in antimicrobial drug use (as defined daily dosages per animal per year, DDDA/Y) and prevalence of resistance in *E. coli* isolates for broilers, pigs, veal calves and dairy cows during a reduction in antimicrobial use from index year 2009 to year 2014 in the Netherlands

	AMU[a] (DDDA/Y)			AMR[a] (%)			Absolute change 2009–14		Relative change 2009–14	
Animal species	AM class	2009	2014	AM agent[b]	2009	2014	AMU[a] (DDDA/Y)	AMR[a] (%)	AMU[a] (DDDA/Y)	AMR[a] (%)
Broilers	total AMU	36.8	15.8	1-PS	87.6	80.6	−21.0	−7.0	−57.1	−8.0
	tetracyclines	5.6	1.7	TET	61.9	42.4	−3.9	−19.4	−69.8	−31.4
	penicillins	14.3	9.9	AMP	73.2	62.1	−4.4	−11.1	−30.5	−15.2
	trimethoprim/ sulphonamides	2.2	1.3	TMP	62.2	44.6	−0.8	−17.6	−37.7	−28.4
				SMX	71.8	52.5				
	amphenicols	0.0	0.0	CHL	23.7	13.5	0.0	−10.2	0.0	−42.9
	fluoroquinolones	0.5	0.2	CIP	57.4	46.4	−0.3	−11.0	−64.7	−19.1
	quinolones	6.7	2.1	NAL	57.4	44.6	−4.5	−12.8	−68.0	−22.3
	3rd/4th-gen. cephalosporins	0.0	0.0	CTX	17.9	2.9	0.0	−15.0	0.0	−83.7
	aminoglycosides	0.0	0.0	STR	67.4	NA	0.0	NA	0.0	NA
				GEN	8.6	6.4		−2.2		−25.9
Pigs	total AMU	20.5	9.5	1-PS	80.4	63.0	−11.0	−17.4	−53.6	−21.6
	tetracyclines	10.7	4.3	TET	67.6	49.2	−6.4	−18.3	−59.4	−27.1
	penicillins	2.8	2.1	AMP	44.9	24.0	−0.7	−21.0	−25.9	−46.6
	trimethoprim/ sulphonamides	3.6	1.3	TMP	53.7	30.9	−2.2	−22.8	−62.6	−42.5
				SMX	61.8	41.3				
	amphenicols	0.0	0.2	CHL	11.5	12.0	0.1	0.5	278.6	4.4
	fluoroquinolones	0.0	0.0	CIP	7.1	0.0	0.0	−7.1	−100.0	−100.0
	quinolones	0.0	0.1	NAL	7.1	0.3	0.0	−6.8	45.8	−96.4
	3rd/4th-gen. cephalosporins	0.1	0.0	CTX	3.7	0.5	−0.1	−3.2	−100.0	−86.3
	aminoglycosides	0.0	0.0	STR	62.5	NA	0.0	NA	0.0	NA
				GEN	3.0	3.6		0.5		17.5
Veal calves	total AMU	33.8	21.2	1-PS	66.1	49.0	−12.7	−17.1	−37.4	−25.9
	tetracyclines	17.8	10.7	TET	59.1	44.5	−7.1	−14.5	−40.0	−24.6
	penicillins	1.5	2.2	AMP	41.5	22.3	0.7	−19.3	44.3	−46.4
	trimethoprim/ sulphonamides	3.6	2.1	TMP	37.4	22.3	−1.5	−15.2	−41.4	−40.5
				SMX	45.0	28.1				
	amphenicols	0.6	1.5	CHL	22.2	13.4	0.9	−8.9	145.2	−39.9
	fluoroquinolones	0.9	0.0	CIP	18.1	6.5	−0.8	−11.6	−97.7	−64.1
	quinolones	0.2	0.5	NAL	18.7	5.8	0.3	−12.9	133.3	−68.9
	3rd/4th-gen. cephalosporins	0.4	0.0	CTX	1.8	1.0	−0.4	−0.7	−100.0	−41.4
	aminoglycosides	0.1	0.3	STR	47.4	NA	0.3	NA	580.0	NA
				GEN	6.4	3.8		−2.7		−41.4
Dairy cattle	total AMU	5.8	3.3	1-PS	23.1	4.9	−2.5	−18.3	−43.0	−79.0
	tetracyclines	0.6	0.4	TET	18.4	3.0	−0.2	−15.4	−37.1	−83.8
	penicillins	2.8	2.0	AMP	11.8	1.5	−0.8	−10.3	−27.4	−87.3
	trimethoprim/ sulphonamides	0.2	0.2	TMP	12.5	0.0	0.0	−12.5	14.3	−100.0
				SMX	16.2	2.6				
	amphenicols	0.0	0.1	CHL	5.9	1.1	0.0	−4.8	100.0	−81.0
	fluoroquinolones	0.1	0.0	CIP	4.5	0.0	−0.1	−4.5	−100.0	−100.0
	quinolones	0.0	0.0	NAL	5.9	0.0	0.0	−5.9	0.0	−100.0

Continued

Table 2. *Continued*

Animal species	AMU[a] (DDDA/Y) AM class	2009	2014	AMR[a] (%) AM agent[b]	2009	2014	Absolute change 2009–14 AMU[a] (DDDA/Y)	AMR[a] (%)	Relative change 2009–14 AMU[a] (DDDA/Y)	AMR[a] (%)
	3rd/4th-gen. cephalosporins	0.8	0.0	CTX	1.5	0.4	−0.8	−1.1	−100.0	−74.6
	aminoglycosides	0.0	0.0	STR	16.9	NA	0.0	NA	0.0	NA
				GEN	5.9	0.4		−5.5		−93.7

AMP, ampicillin; TET, tetracycline; SMX, sulfamethoxazole; TMP, trimethoprim; CIP, ciprofloxacin; NAL, nalidixic acid; CHL, chloramphenicol; CTX, cefotaxime; GEN, gentamicin. STR, streptomycin was only used for testing during 2007–13 and calculations for changes in 2009–14 are not applicable (NA).

[a]AMU, antimicrobial use; AMR, antimicrobial resistance. Decreasing changes are gradually shaded in grey (the darker, the bigger the decrease).

[b]AM, antimicrobial agent; 1-PS, 1 – [pan-susceptibility], resistance to at least one of the agents of the susceptibility testing panel (i.e. 1−proportion of fully susceptible isolates).

with homologous antimicrobial use were not statistically significant (Figure 2a and b, Table 3).

In dairy cattle, the antimicrobial use–resistance associations were the strongest (i.e. higher ORs), but they were not statistically significant (Figure 2a and b, Table 3).

Occasionally, the models in the different species generated negative estimates which we deemed are likely implausible (i.e. increased antimicrobial use associated with reduced resistance). These estimates probably resulted from the intrinsic nature of our data (e.g. potential misclassifications) or from having fewer data points (e.g. for streptomycin, which was tested only during 2007–13, ORs$<$1 in broilers) (Figure 2a and b, Table 3).

Predicting resistance prevalence associated with an 80% reduction in antimicrobial use

Predicted resistance levels for 2016 in broilers remained similar to the observed ones in 2014; only ciprofloxacin and ampicillin resistance were expected to be reduced by ∼3%–4% but in some cases resistance was expected to increase (e.g. for tetracycline and cefotaxime) (Table 4). In pigs and veal calves, predictions were the most optimistic; 1 − [pan-susceptibility] and resistance to the most commonly used antimicrobials were projected to decrease by ∼5%–9% in pigs and by ∼14%–28% in veal calves. In dairy cattle, 1 − [pan-susceptibility] was projected to decrease by ∼3% (Table 4).

Discussion

Our results suggest that a reduction in the use of antibiotics during the past 5 years in the Netherlands resulted in lowered *E. coli* resistance levels in some livestock industries. Generally positive and (borderline) statistically significant antimicrobial use–*E. coli* resistance relationships were derived, but the associations clearly differed between animal sectors. A further decrease in the use of these drugs was projected to have a clear impact, resulting in lower predicted levels of *E. coli* resistance, in the pig and veal calf industry. However, the relationships in broilers and dairy cattle were weaker and changes in *E. coli* resistance were projected to be minor. Multiresistant isolates were very common in all animal sectors and usage history and co-selection of phenotypes might explain to a large extent the observed patterns.

We focused on *E. coli* for various reasons. This bacterium is a widely used indicator of Gram-negative species incorporated in resistance surveillance systems, thus continuous data over several years were readily available.[19,26] Moreover, *E. coli* is highly abundant in the intestinal tract of humans and animals and is an important vector for transmission of resistance genes between bacterial populations.[27]

The use of DDDA/Y is a refined way of reporting antibiotic consumption, which has been recommended by the European Surveillance of Veterinary Antimicrobial Consumption (ESVAC).[13,20] It enables the reporting of consumption by animal species and by antibiotic class, and it is considered as a better proxy of exposure since it accounts for the long-acting properties of some antimicrobials. Sampling in the monitoring programme was done mainly at the slaughterhouse level. Thus, resistant microorganisms should theoretically reflect antimicrobial use patterns and management practices as a whole during the life of these animals. However, it should be noted that antimicrobial consumption also occurs throughout the whole production chain in other animal groups (e.g. sows, boars, grandparent broilers) that are not directly represented by this sampling strategy. Sample size was sufficient to provide an estimate of the resistant *E. coli* population in each animal species of the entire country and, for representativeness, animals were sampled in all months of the year to account for possible seasonal effects.[19] Nonetheless, it should be noted that this sampling scheme is inherently insensitive to detecting resistance at the individual animal level and the resistance measure might not provide as sharp a picture of the situation at individual farms.[19,28] This could be the reason for the drop in prevalence observed in veal calves after 2011 (Figure 1), when the sampling scheme changed from farms to slaughterhouses. In the case of dairy cattle, the sharp increase in resistance observed in 2009 (Figure 1) was not fully explained but is probably attributable to the smaller sample taken in that year (n=136), which made it less representative.[19]

Antimicrobial use differed quantitatively and qualitatively by animal sector and this appeared to drive the variation observed in resistance patterns (Table 1). For instance, quinolone and fluoroquinolone resistance levels were exceptionally high in

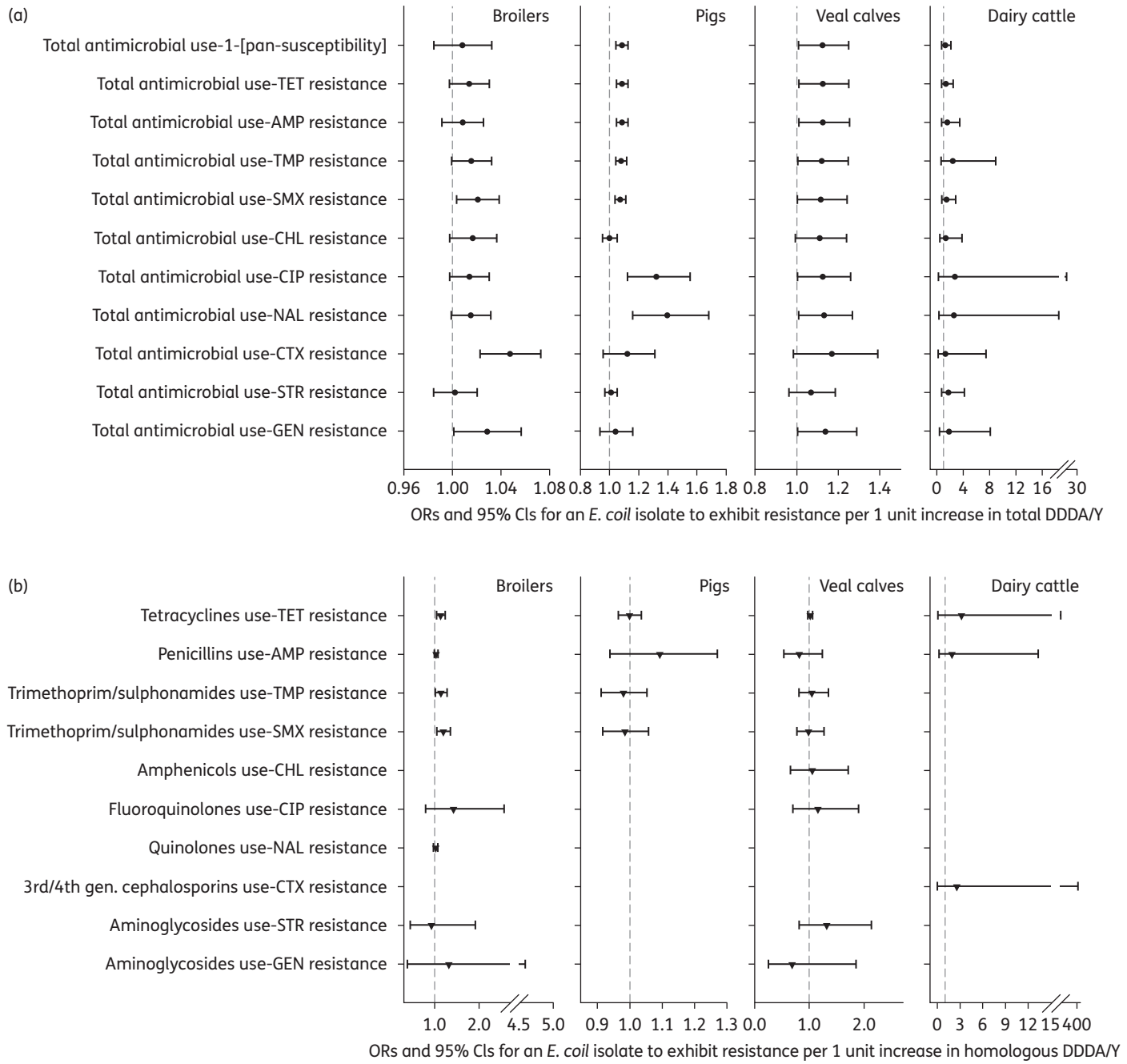

Figure 2. Probabilities (ORs and 95% CIs) for an *E. coli* isolate to exhibit resistance per 1 DDDA/Y increase in total antimicrobial use (a, filled circles) or in homologous antimicrobial class use (b, filled triangles) in different food-producing animal sectors in the Netherlands 2004–14. Results from the two multivariate random-effects generalized linear mixed model (logistic regression) fitted with total antimicrobial use as determinant (a) and homologous antimicrobial use (b; only classes with DDDA/Y > 0.5 in all years were modelled) (Table 3). The plotted estimates for veal calves originated from the model not accounting for change in sampling strategy. The dotted vertical reference line is used to assess significance (i.e. statistically significance at 95% CI if OR and CI are >1). AMP, ampicillin; TET, tetracycline; SMX, sulfamethoxazole; TMP, trimethoprim; CIP, ciprofloxacin; NAL, nalidixic acid; CHL, chloramphenicol; CTX, cefotaxime; STR, streptomycin (only tested from 2007 to 2013); GEN, gentamicin. 1 − [pan-susceptibility] refers to isolates resistant to at least one of the agents of the susceptibility testing panel (i.e. 1 − proportion of fully susceptible isolates).

broilers as a result of the higher use of quinolones as compared with the other animal species. Our results reconfirm the potential importance of co-selection (i.e. an antimicrobial selects for resistance to another antimicrobial) in the emergence and perpetuation of this problem. Multiresistance commonly involved the most widely administered antimicrobials and with several decades of prior usage (penicillins, tetracyclines and trimethoprim/sulphonamides). Multiple resistance can perpetuate for

Table 3. Probabilities (ORs and 95% CIs) for an *E. coli* isolate to test resistance per 1 DDDA/Y increase in antimicrobial use for broilers, slaughter pigs, veal calves and dairy cows in the Netherlands 2004–14[a]

	Model with total antimicrobial use as determinant			Model with homologous antimicrobial use as determinant		
Animal species	AMU[b]	AMR[c]	OR and 95% CI	AMU[b]	AMR[c]	OR and 95% CI[d]
Broilers	Total AMU	1-PS	1.01 (0.98–1.03)			NA
		TET	1.01 (1.00–1.03)	tetracyclines	TET	1.13 (1.04–1.24)*
		AMP	1.01 (0.99–1.03)	penicillins	AMP	1.03 (0.99–1.07)
		TMP	1.02 (1.00–1.03)*	trimethoprim/sulphonamides	TMP	1.14 (1.01–1.28)*
		SMX	1.02 (1.00–1.04)*		SMX	1.19 (1.05–1.35)*
		CHL	1.02 (1.00–1.04)	amphenicols	CHL	NC
		CIP	1.01 (1.00–1.03)	fluoroquinolones	CIP	1.42 (0.79–2.57)
		NAL	1.02 (1.00–1.03)	quinolones	NAL	1.02 (0.97–1.07)
		CTX	1.05 (1.02–1.07)*	3rd/4th-gen. cephalosporins	CTX	NC
		STR	1.00 (0.98–1.02)	aminoglycosides	STR	0.93 (0.45–1.92)
		GEN	1.03 (1.00–1.06)*		GEN	1.32 (0.38–4.59)
Pigs	Total AMU	1-PS	1.08 (1.04–1.13)*			NA
		TET	1.08 (1.05–1.12)*	tetracyclines	TET	1.00 (0.96–1.04)
		AMP	1.08 (1.05–1.13)*	penicillins	AMP	1.09 (0.94–1.27)
		TMP	1.08 (1.04–1.12)*	trimethoprim/sulphonamides	TMP	0.98 (0.91–1.05)
		SMX	1.07 (1.04–1.11)*		SMX	0.99 (0.92–1.06)
		CHL	1.00 (0.95–1.05)	amphenicols	CHL	NC
		CIP	1.32 (1.12–1.55)*	fluoroquinolones	CIP	NC
		NAL	1.40 (1.16–1.68)*	quinolones	NAL	NC
		CTX	1.12 (0.96–1.31)	3rd/4th-gen. cephalosporins	CTX	NC
		STR	1.01 (0.97–1.05)	aminoglycosides	STR	NC
		GEN	1.04 (0.93–1.16)		GEN	NC
Veal calves (model not accounting for change in sampling strategy)	Total AMU	1-PS	1.12 (1.01–1.25)*			NA
		TET	1.12 (1.01–1.25)*	tetracyclines	TET	1.02 (0.97–1.06)
		AMP	1.13 (1.01–1.25)*	penicillins	AMP	0.81 (0.53–1.24)
		TMP	1.12 (1.00–1.25)*	trimethoprim/sulphonamides	TMP	1.05 (0.81–1.35)
		SMX	1.12 (1.00–1.25)*		SMX	0.98 (0.77–1.26)
		CHL	1.11 (0.99–1.24)	amphenicols	CHL	1.06 (0.65–1.71)
		CIP	1.12 (1.00–1.26)	fluoroquinolones	CIP	1.29 (0.44–3.79)
		NAL	1.13 (1.01–1.27)*	quinolones	NAL	NC
		CTX	1.17 (0.99–1.39)	3rd/4th-gen. cephalosporins	CTX	NC
		STR	1.07 (0.96–1.19)	aminoglycosides	STR	1.34 (0.84–2.15)
		GEN	1.14 (1.00–1.29)*		GEN	0.71 (0.27–1.89)
Veal calves (model accounting for change in sampling strategy)[e]	Total AMU	1-PS	0.99 (0.93–1.06)			NA
		TET	1.00 (0.94–1.06)	tetracyclines	TET	1.01 (0.97–1.17)
		AMP	1.00 (0.94–1.06)	penicillins	AMP	0.87 (0.56–1.35)
		TMP	0.99 (0.93–1.06)	trimethoprim/sulphonamides	TMP	1.03 (0.79–1.34)
		SMX	0.99 (0.93–1.05)		SMX	0.96 (0.74–1.24)
		CHL	0.99 (0.93–1.05)	amphenicols	CHL	1.06 (0.64–1.74)
		CIP	1.00 (0.94–1.07)	fluoroquinolones	CIP	1.23 (0.75–2.03)
		NAL	1.01 (0.94–1.08)	quinolones	NAL	NC
		CTX	1.05 (0.95–1.16)	3rd/4th-gen. cephalosporins	CTX	NC
		STR	0.95 (0.89–1.01)	aminoglycosides	STR	1.47 (0.90–2.40)
		GEN	1.02 (0.95–1.09)		GEN	0.68 (0.27–1.70)
Dairy cattle	Total AMU	1-PS	1.30 (0.78–2.17)			NA
		TET	1.39 (0.77–2.51)	tetracyclines	TET	3.16 (0.08–119.9)
		AMP	1.57 (0.71–3.49)	penicillins	AMP	1.90 (0.27–13.32)
		TMP	2.45 (0.67–8.95)	trimethoprim/sulphonamides	TMP	NC

Continued

Table 3. *Continued*

Animal species	Model with total antimicrobial use as determinant			Model with homologous antimicrobial use as determinant		
	AMU[b]	AMR[c]	OR and 95% CI	AMU[b]	AMR[c]	OR and 95% CI[d]
		SMX	1.46 (0.74–2.9)		SMX	NC
		CHL	1.35 (0.47–3.85)	amphenicols	CHL	NC
		CIP	2.72 (0.29–25.57)	fluoroquinolones	CIP	NC
		NAL	2.62 (0.37–18.5)	quinolones	NAL	NC
		CTX	1.30 (0.23–7.46)	3rd/4th-gen. cephalosporins	CTX	2.57 (0.02–416.46)
		STR	1.77 (0.74–4.21)	aminoglycosides	STR	NC
		GEN	1.85 (0.42–8.11)		GEN	NC

An asterisk indicates a significant association at the 95% CI level. AMP, ampicillin; TET, tetracycline; SMX, sulfamethoxazole; TMP, trimethoprim; CIP, ciprofloxacin; NAL, nalidixic acid; CHL, chloramphenicol; CTX, cefotaxime; STR, streptomycin (only tested from 2007 to 2013); GEN, gentamicin.
[a]Two multivariate random-effects generalized linear mixed model (logistic regression) are fitted per animal species; one with total antimicrobial use and the other with homologous antimicrobial usage as determinants. Model outcomes are the frequencies of resistant isolates over total number of isolates. Resistance phenotypes to each antimicrobial agent (and 1 − [pan-susceptibility]) are indicated in the model as an explanatory variable and its interaction with antimicrobial use (DDDA/Y total and disaggregated by classes) differentiates the outcomes. ORs presented in the table are extracted from the interaction term.
[b]AMU, antimicrobial use. Determinant of the model.
[c]AMR, antimicrobial resistance. 1-PS, 1 − [pan-susceptibility], resistance to at least one of the agents of the susceptibility testing panel (i.e. 1 − proportion of fully susceptible isolates).
[d]Homologous resistance was only modelled in the classes with DDDA > 0.5 in all years (NA, not applicable; NC, not computed).
[e]A variable indicating the different sampling strategy (until 2011 in farms and from 2012 in slaughterhouses) was included in the model for adjustment of the estimates.

years, even with decreasing or no use of antibiotics, since resistance genes are often assembled in complex genetic vectors containing other resistance genes.[27,29] This is the most likely explanation for the observed moderate to high levels of resistance to chloramphenicol and streptomycin, when use of these drugs was virtually zero (Figure 1). Resistance to more recently introduced drugs (around the 1990s), such as fluoroquinolones and third-generation cephalosporins, occurred less frequently and was often associated with multiresistance (Table 1). Comparable findings have been described elsewhere.[29]

This study suggests that curbing *E. coli* resistance rates in some livestock sectors is to a certain extent possible by reducing the use of antimicrobials. Specifically, cefotaxime and ciprofloxacin resistance was even more dramatically reduced in relative terms (Table 2), which we deemed to be a product of the restriction in usage of third-/fourth-generation cephalosporins and fluoroquinolones.[30,31] These findings suggest that, in *E. coli*, resistance to antibiotics with shorter usage history could be more rapidly reverted. Nonetheless, non-monotonic trends (e.g. reduction or suppression of antimicrobials related to both increased and decreased resistance over different time periods) have been described on several occasions for these drugs.[29]

We found positive antimicrobial use–resistance dose–response relationships that varied in magnitude and statistical significance by association and by animal sector (Figure 2 and Table 3). Similar associations have been demonstrated, especially in pigs and veal calves, but rarely based on longitudinal data and at the national level.[1,3,6,32,33] In a recent study, a direct antimicrobial use–resistance correlation at a supranational level was described, but findings were compromised by the cross-sectional nature of the associations and the lack of data per individual animal sectors.[2] We found the strongest usage–resistance relationships in slaughter pigs and veal calves, followed by the broilers (sectors with a relatively high use of antibiotics). In dairy cattle no statistically significant relationships were found. These observations might reflect important differences between animal industries in the structure, regimens of drug prescription and durations of the production cycle (broilers < veal calves < pigs < dairy cattle). In veal production, greater quantities of antimicrobials are applied cycle after cycle, and the frequency of animal replacement is lower than in other sectors. Pig production is an age-segregated system with continuous replacement, but with more stringent biosecurity conditions. Broilers are produced in a highly integrated system, where few companies control the supply of animals; they are also raised under highly confined conditions and receive more broad-spectrum drugs such as quinolones (i.e. more multiresistance). Administration of antimicrobials in dairy cows is usually more limited and on an individual basis, which could explain the lack of significant associations in our models. Despite all possible hypotheses, the reasons behind the low impact of the policy to reduce antimicrobial use in the broiler and dairy sectors requires further investigation.

We also unravelled differences between types of resistance (Figure 2 and Table 3). Exposure–response slopes were often steeper for newer drugs (i.e. higher ORs for third-/fourth-generation cephalosporins in broilers and veal calves and higher ORs for fluoroquinolones in pigs and dairy cattle), but this needs cautious interpretation; baseline resistance levels were lower for these drugs, which might lead to larger estimates, although it also probably explained by a more rapidly reverted resistance to these

Table 4. Predicted prevalences (%) of resistance in *E. coli* isolates for the year 2016 in the different food-producing animal sectors if their total antimicrobial use was decreased by 80% from index year 2009[a]

Animal species	AMR[b]	Observed resistance prevalence (%) in 2014[c]	Predicted resistance prevalence (%) in 2016	Predicted absolute change prevalence (%) between 2014 and 2016[d]
Broilers	1-PS	80.6	82.3	1.6
	TET	42.4	44.0	1.5
	AMP	62.1	58.0	−4.1
	TMP	44.6	44.6	0.0
	SMX	52.5	53.0	0.4
	CHL	13.5	13.3	−0.2
	CIP	46.4	43.0	−3.4
	NAL	44.6	42.7	−1.9
	CTX	2.9	4.3	1.4
	STR[c]	58.1	55.3	−2.8
	GEN	6.4	4.3	−2.1
Pigs	1-PS	63.0	53.7	−9.3
	TET	49.2	40.8	−8.4
	AMP	24.0	16.0	−8.0
	TMP	30.9	25.7	−5.2
	SMX	41.3	33.6	−7.8
	CHL	12.0	11.7	−0.3
	CIP	0.0	0.1	0.1
	NAL	0.3	0.0	−0.2
	CTX	0.5	0.3	−0.2
	STR[c]	50.2	52.4	2.2
	GEN	3.6	1.5	−2.1
Veal calves[e]	1-PS	49.0	21.0	−28.0
	TET	44.5	19.0	−25.5
	AMP	22.3	7.7	−14.6
	TMP	22.3	8.0	−14.2
	SMX	28.1	11.7	−16.4
	CHL	13.4	4.9	−8.5
	CIP	6.5	2.3	−4.2
	NAL	5.8	2.0	−3.8
	CTX	1.0	0.1	−0.9
	STR[c]	29.3	23.8	−5.5
	GEN	3.8	0.7	−3.0
Dairy cattle	1-PS	4.9	1.5	−3.4
	TET	3.0	0.8	−2.2
	AMP	1.5	0.3	−1.2
	TMP	0.0	0.0	0.0
	SMX	2.6	0.5	−2.1
	CHL	1.1	0.3	−0.9
	CIP	0.0	0.0	0.0
	NAL	0.0	0.0	0.0
	CTX	0.4	0.1	−0.3
	STR[c]	1.1	0.2	−0.9
	GEN	0.4	0.1	−0.3

AMP, ampicillin; TET, tetracycline; SMX, sulfamethoxazole; TMP, trimethoprim; CIP, ciprofloxacin; NAL, nalidixic acid; CHL, chloramphenicol; CTX, cefotaxime; STR, streptomycin (only tested from 2007 to 2013); GEN, gentamicin.

[a]Predictions for percentage of resistance obtained for year 2016 and the total antimicrobial use corresponding to an 80% reduction from 2009. Only the multivariate random-effects generalized linear mixed models with total antimicrobial use as determinant were used.

[b]AMR, antimicrobial agent resistance. 1-PS, 1 − [pan-susceptibility], resistance to at least one of the agents of the susceptibility testing panel (i.e. 1 − proportion of fully susceptible isolates).

[c]The observed prevalence of resistance for STR (streptomycin) indicated in the table are for the year 2013 (the last year in which STR was included in the susceptibility testing panel).

[d]Decreasing changes are gradually shaded in grey (the darker, the bigger the decrease).

[e]In veal calves only the model not accounting for the change in sampling strategy was used to obtain the prediction.

antimicrobials. Moreover, total antimicrobial use was more significantly related to resistance phenotypes than homologous usage, showing the potential importance of co-selection of resistance. This means that if a reduction in resistance to a specific antimicrobial is intended, a reduction in all of the antimicrobials co-selecting for the same resistance is essential. Nonetheless, and regardless the significance, direct selection also played a role since some homologous associations had a greater effect.

A further decrease in antimicrobial use was predicted to result in more decreased resistance in the veal calf and pig sectors. In broilers, resistance trends seemed to level off (Table 4); a number of potential explanations exist. It is clear that use of antimicrobials contributes significantly to resistance emergence, but additional forces drive this process, such as movement of carrier animals between premises, transmission from the top of pyramidal production systems, keeping animals in close confinement, biosecurity and hygiene conditions, etc.[34–37] Moreover, illegal use of ceftiofur was noticed up to March 2010 at broiler hatcheries; this use was not recorded and reported, but was deemed to increase levels of resistance to third-generation cephalosporins.[19]

The data for this study came from publicly available reports. However, limiting factors existed in terms of interpretation and the level of detail. Our data were ecological, that is to say, antimicrobial use and resistance were evaluated at country level and not in corresponding and equal epidemiological units (e.g. farm level). This leads to potential misclassification of exposure

to antimicrobials, which might be especially important in heterogeneous sectors such as veal calf production. Causality in this study strongly relied on the temporal and geographical link. An extra limitation arising from the level of aggregation of our data was the impossibility to account for other farm-level determinants of resistance and to adjust associations for these.[6,38] Notwithstanding these constraints, we consider the data resolution to have been sufficient for the purpose of evaluating the nationwide programme. A methodological limitation was that our models were suited for well-established and long-standing relatively high levels of antimicrobial use and resistance; in order to obtain reliable estimates and model convergence, some homologous associations could not be studied when antimicrobials were used in very low quantities.

Conclusions

Recent Dutch policies reducing the total veterinary use of antimicrobials, and restricting the use of critically important antimicrobials, appear to have reduced (and are projected to further curb) *E. coli* resistance levels in veal calves and slaughter pigs. The impact on dairy cattle and broilers was, however, minor. Epidemiological evidence highlights the importance of a better understanding on the co-selection of resistance. Additional interventions need to be evaluated in future studies.

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Transparency declarations

None to declare.

Supplementary data

Further information on antimicrobial use and resistance data and Tables S1–S4 are available as Supplementary data at *JAC* Online (http://jac.oxfordjournals.org/).

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