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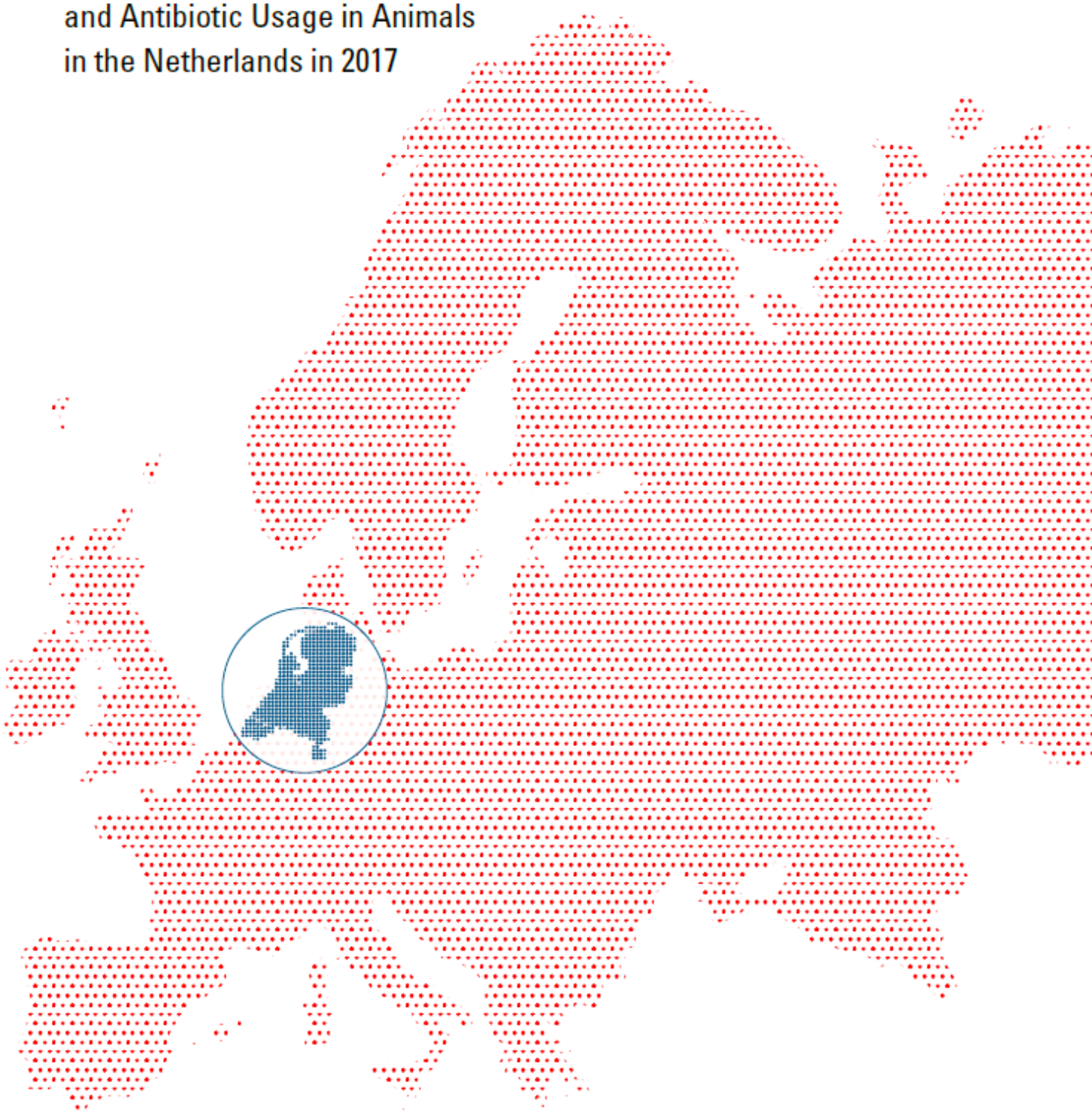
Monitoring of Antimicrobial Resistance and Antibiotic Usage in Animals in the Netherlands in 2017



National Institute for Public Health and the Environment
Ministry of Health, Welfare and Sport

Netherlands Food and Consumer Product Safety Authority
Ministry of Agriculture, Nature and Food Quality

WAGENINGEN UNIVERSITY & RESEARCH

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Monitoring of Antimicrobial Resistance
and Antibiotic Usage in Animals in the Netherlands
in 2017

June 2018

Colophon

This report is published under the acronym MARAN-2018 by Wageningen Bioveterinary Research (WBVR) in collaboration with the Food and Consumer Product Safety Authority (NVWA), the National Institute for Public Health and the Environment (RIVM) and the Netherlands Veterinary Medicines Institute (SDa). The information presented in MARAN-2018 is based on total sales data and animal specific usage of antimicrobial agents in animal husbandry and the occurrence of antimicrobial resistance in bacteria of animal origin and of relevance to public health.
MARAN-2018 is published in a combined back-to-back report with NETHMAP-2018. The combined report is available on the website of WBVR at www.wur.nl More detailed information on the usage of antibiotics per animal species is available on the website of the Netherlands Veterinary Medicines Institute (www.autoriteitdiergeneesmiddelen.nl).
MARAN-2018 can be ordered from the secretariat of WBVR, p/a Houtribweg 39, 8221 RA Lelystad, The Netherlands.

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Contents

1 Summary

Antibiotic Usage

Sales of antimicrobial veterinary medicinal products (AVMP's) in 2017 (181 tonnes) showed an increase of 3% compared to 2016 (176 tonnes). In 2016, sales barely covered monitored and extrapolated use; reasons for the increase of sales in 2017 could be an increase in stock (catching up) and increased use in growing unmonitored sectors.

In most sectors, veal valves, pigs, broilers and turkeys, a reduction in consumption has been realized. In dairy cows and other cattle a small increase in consumption is noted. The calculation of consumption is based on national conversion factors (DDDA's) of authorized drugs. Maximal transparency has been created since 2011 through monitoring antibiotics use by veterinarians and farmers.

The use of antibiotics of critical importance to human health care (especially cephalosporins of 3rd and 4th generation) is reduced to an absolute minimum, even in the unmonitored sectors. Import of these AVMP's from other EU member states is not monitored in sales data, but if used in the monitored animal sectors, veterinarians are obliged to report these VMP's.

Antimicrobial resistance

In 2017 *S.* Enteritidis (25.6%) followed by *S.* Typhimurium (15.9%) together with the monophasic variant of Typhimurium: *S.* enterica subspecies *enterica* 1,4,[5],12:i:- (15.7%), were most frequently isolated from humans suffering from salmonellosis. In pigs, the monophasic variant of *S.* Typhimurium dominated. In cattle, *S.* Typhimurium and *S.* Dublin were most commonly isolated. In poultry (including poultry products and broilers), the number of *S.* Paratyphi B var. Java was equal to 2016. The most isolated serovar in poultry meat in 2017 was *S.* Heidelberg. The highest proportions of resistance were observed in the *S.* Heidelberg, monophasic *S.* Typhimurium and in *S.* Kentucky, and to a lesser extent in *S.* Typhimurium. Ciprofloxacin resistance was most common amongst isolates from humans and poultry. Predominant serovars were *S.* Kentucky (81.3% resistant), *S.* Infantis (26.2%) and Enteritidis (21.5%).

In 2017, the proportions cefotaxime resistant (MIC > 0.5 mg/L) ESBL suspected *Salmonella* isolates was 1.8% concerning seven different serovars, isolated from human samples. Cefotaxime resistance was

detected in 67.6% of the *Salmonella* isolates obtained from (outside EU) imported poultry products. No cefotaxime resistant isolates were found in fresh meat from Dutch retail (produced within EU). No carbapenemase producing *Salmonella* were found in 2017.

Proportions of resistance in *C. jejuni* from caecal samples of broilers and meat thereof were traditionally high for quinolones and tetracycline and did not substantially change in 2017, compared to 2016. Resistance to macrolides was rarely detected in isolates from livestock and humans and almost exclusively found in *C. coli* isolates from broilers and pigs. Overall, resistance proportions were higher in *C. coli* than in *C. jejuni* isolates.
Ciprofloxacin resistance in *Campylobacter* isolates from human patients is still high (with an increase in 2017), which is a concern for public health. Resistance to erythromycin, first choice antibiotic in human medicine for campylobacteriosis, remained low. For *C. jejuni* and *C. coli* from human patients, resistance proportions were higher for all three antimicrobials tested in travel related infections compared to domestically acquired campylobacteriosis.

Proportions of resistance to ampicillin, sulfamethoxazole and trimethoprim in human STEC O157 isolates were somewhat higher in 2017, compared to 2016 (10.7% to 16.1% for ampicilline, from 14.7% to 16.1% for sulfamethoxazole, and from 8.0% to 14.5% for trimethoprim). There is an increasing tendency for resistance against these antimicrobials since 2009. Resistance to the quinolones (ciprofloxacin and nalidixic acid) was detected in 3.2% of human STEC O157 isolates. For the first time since seven years one cefotaxime resistant, ESBL-producing isolate was detected.

In 2017, resistance proportions of indicator *E. coli* in caecal samples showed a tendency to decrease in broilers, to stabilize in pigs, and showed a slight increase in veal calves. In dairy cattle the resistance proportions remained at a constant low level. As in former years, resistance proportions in *E. coli* from chicken and turkey meat, were substantially higher than in pork and beef. The proportion of *E. coli* isolates resistant to third-generation cephalosporins was low in faecal samples from broilers and pigs and they were not detected in dairy cattle and veal calves. Although resistance to fluoroquinolones is decreasing, it was still commonly present in indicator *E. coli* from caecal samples of broilers and meat thereof. Among indicator *E. coli* from animals and meat, resistance levels to ampicillin, tetracycline, sulfamethoxazole and trimethoprim were still high in broilers, pigs, veal calves and chicken and turkey meat. Levels of resistance in *E. coli* from caecal samples of rosé veal calves were substantially lower than those from white veal calves for almost all antibiotics tested.

Within the randomly isolated indicator *E. coli* in caecal samples from broilers a continuous low proportion of ESBL/AmpC-producing *E. coli* was observed in the last five years (<3%) and this was confirmed in 2017 (1.7%). No ESBL/AmpC-producing indicator *E. coli* were detected by random isolation in faecal samples from pigs, veal calves and dairy cattle. Selective culturing in livestock faeces showed a further decrease in the prevalence (% of animal carriers) of ESBL/AmpC-producing *E. coli* in broilers. For the second year in a row, an increase was observed in white and rosé veal calves carrying ESBL/AmpC-producing *E. coli*, using selective culturing. 2017 was the first year a higher prevalence was recorded in veal calves than in broilers (36.7% vs 32.6%).
The most prevalent ESBL/AmpC gene was $bla_{\text{CTX-M-1}}$ in all animal species. $bla_{\text{CTX-M-15}}$ was found frequently in veal calves and dairy cows (30%). $bla_{\text{CMY-2}}$ in broilers (25%), followed by $bla_{\text{SHV-12}}$, $bla_{\text{TEM-52c}}$ and $bla_{\text{CTX-M-14}}$.

A comparable gene distribution was observed in corresponding meat samples. The overall prevalence of ESBL/AmpC-producing *E. coli* in meat in 2017 was 9.6%. After three years of decreasing prevalence (67% to 24% in 2014-2016), in 2017 31.6% of fresh chicken meat samples were found positive, resulting in a similar prevalence as in broilers (32.6%). Imported chicken meat was more frequently positive (56.1%). Also lamb and veal meat were more frequently found positive than in previous years.
The proportion of human ESBL/AmpC-producing *Salmonella* in 2017 was 1.8%, confirming a continuous low level (≤2%) since 2014. Most represented ESBL/AmpC genes were $bla_{\text{CTX-M-14b}}$, generally associated with S. Kentucky, $bla_{\text{CTX-M-9}}$ in S. Typhimurium, and $bla_{\text{CMY-2}}$ in S. Typhimurium and S. Agona. The majority (84%) of ESBL/AmpC-producing *Salmonella* from humans were highly multidrug resistant (5-8 antibiotics).

No carbapenemase-producing *Enterobacteriaceae* were detected in active surveillance in livestock. Only $bla_{\text{OXA-48-like}}$ genes were detected in six samples (three broilers, two slaughter pigs and one dairy cow) and all associated with *Shewanella* spp..

In an ongoing prospective study of faecal samples of companion animals one dog was found to be carrier of *E. coli* carrying $bla_{\text{OXA-48}}$. This was the first time such a carbapenemase producing isolate was detected in a dog in the Netherlands. Molecular analysis of the isolate is ongoing but preliminary analysis suggests that the $bla_{\text{OXA-48}}$ gene is transferable because it is located on a mobile element.

Colistin resistance gene *mcr-1* was identified at a low-level in *E. coli* from livestock (1.2%) and at higher levels in retail meat from chicken (7.7%), but not in *Salmonella*.

It can be concluded that the sales of antibiotics for animals remained stable compared to 2016. In 2017 a clear reduction in antibiotic use was only observed in broilers and turkeys, while in use pigs and veal calves showed a small reduction and use in dairy cattle showed a small increase. The use of antibiotics of critical importance to human health care (especially cephalosporins of 3rd and 4th generation) remains to be very minimal.
The usage data are to a large extend reflected in the resistance data of 2017 where proportions of resistant *E. coli* stabilized in pigs compared to constant decreasing tendencies since 2009. In veal calves the resistant proportions have been stable since 2012 and showed a slight increase in 2017. In broilers the continuous reduction in use resulted in an ongoing decrease in proportions of resistant *E. coli* for most antibiotic classes tested. Also the concentration of ESBL/AmpC-producing *E. coli* in broiler faeces and on poultry meat was again lower than in previous years. In contrast to broilers, in 2017 the prevalence of ESBL-carriers again increased in both white and rosé veal calves. This shows that the measures implemented in Dutch livestock production to reduce the overall antibiotic use and to stop the use of 3rd-generation cephalosporins have been effective in reducing ESBL/AmpC-contamination of food-products. But, they have not been sufficiently effective in the veal calf sector, where antimicrobial resistance remained stable and ESBL occurrence increased. As in previous years carbapenemase producing *Enterobacteriaceae* or the colistin resistance gene *mcr-1*, were not detected or found at low levels, respectively.

2 Usage of antibiotics in animal husbandry in the Netherlands

2.1 Total sales of veterinary antibiotics in the Netherlands 2017

2.1.1 Analysis of sales data

FIDIN, the federation of the Dutch veterinary pharmaceutical industry, provided sales data for all antimicrobial veterinary medicinal products (AVMP's) on package level sold in 2017 in the Netherlands, as extracted from the Vetindex and supplemented with AVMP's data of non-FIDIN members.
These data are estimated to cover approximately 98% of all sales in the Netherlands. Actual use can be different from the quantities sold due to stock piling and cross border use. Monitored use in the major livestock farming sectors (pigs, broilers, turkey, veal calves, dairy- and other cattle) covered 90.6% of sales in 2017.
The European Medicines Agency (EMA) collects harmonised systemic antibiotic usage data based on overall sales of veterinary antimicrobial agents through the European Surveillance of Veterinary Antimicrobial Consumption (ESVAC) project since September 2009. Sales figures from 1999 to 2008 were recalculated and adjusted according to the ESVAC protocol. Data as from 2011 are calculated according to the SDa method for all AVMP's, which means only active base substance mass (excluding mass of salts and esters) is calculated, including (unlike the ESVAC reports) topical applications like ointments, eye drops and sprays. The sales data in this report involves total sales, for all animals, not stratified by animal species. Detailed information about antibiotic usage by animal species in the Netherlands is reported on in a following paragraph.

The average number of food-producing animals present in the Dutch livestock farming sector (pigs, poultry, veal calves, other cattle, sheep, goats, rabbits) shows annual variations (Table ABuse01).
The goat sector involves for 70% dairy goats, and has grown since 2010, and is now half of the sheep sector.

Dairy cattle experienced a major decrease in number of animals because of the phosphate production limitations after the increase of the preceding two years which occurred as a result of the abandoning

of milk quota. With the exception of piglets (and as a result the mass of the pig sector as a whole) and goats, all major production sectors showed a decrease in numbers of animals, while the mass of sold antimicrobial substances increased with 3% in 2017 compared to 2016.

Table ABuse01 Trends in livestock in the Netherlands in numbers (thousands); (Source: poultry and veal calves CBS, other Eurostat).

Number of animals x1000	2009	2010	2011	2012	2013	2014	2015	2016	2017
Piglets (less than 20 kg)	4,809	4,649	4,797	4,993	4,920	5,115	5,408	4,986	5,522
Sows	1,100	1,098	1,106	1,081	1,095	1,106	1,053	1,022	1,066
Fattening pigs	6,199	6,459	6,200	4,189	4,209	4,087	4,223	4,140	3,967
Other pigs	2,100	2,040	2,021	1,841	1,789	1,765	1,769	1,733	1,741
Turkeys	1,060	1,036	990	827	841	794	863	762	671
Broilers	52,323	54,367	57,811	43,912	44,242	47,020	49,107	48,378	48,237
Other poultry	46,383	48,218	40,442	52,356	54,345	56,924	58,636	57,172	56,947
Veal calves	886	921	906	908	925	921	909	956	953
Other cattle	3,112	3,039	2,993	3,045	3,064	3,230	3,360	3,353	3,082
Dairy cattle	1,562	1,518	1,504	1,541	1,597	1,610	1,717	1,794	1,665
Sheep	1,091	1,211	1,113	1,093	1,074	1,070	1,032	1,032	1,015
Goats	374	353	380	397	413	431	470	500	533
Fattening rabbits	271	260	262	284	270	278	333	318	300
Dows	41	39	39	43	41	43	48	45	43

2.1.2 Trends in total sales

Figure ABuse01 and Table ABuse02 show the trends in the total sales of antibiotics licenced for therapeutic use in animals in the Netherlands. Total sales decreased by 63.38 % over the years 2009-2017, the Governmental 70% reduction goal has not been reached yet.

Sales of AVMP's in 2017 (181 tonnes) showed an increase of 3% compared to 2016 (176 tonnes). In 2016, sales barely covered monitored and extrapolated use; reasons for the increase of sales could be an increase in stock (catching up) and increased use in growing unmonitored sectors.

As demonstrated in Figure ABuse02 some groups of antimicrobials show a fluctuating pattern over the years, with an overall decreasing tendency, and some variation from year to year (penicillins, tetracyclines, aminoglycosides and cephalosporins of 1st and 2nd generation). A steady decrease over the years is noted for fixed combinations (mainly mastitis injectors), and the critically important antimicrobials fluoroquinolones, polymyxins, cephalosporins of 3rd and 4th generation, and for trimethoprim/sulfonamides (-13% in 2017 compared to 2016). Sales of amphenicols dropped with 4% in 2017 after increases in earlier years. Also sales of 1st and 2nd generation cephalosporins (-15%) decreased. The sales of quinolones increased (+3%), other antimicrobials (mainly metronidazole and

bacitracin) and macrolides increased each with 5%. Pleuromutilins sales increased with 20%.

Figure ABuse01 Antimicrobial veterinary medicinal product sales 1999-2017 in kg (thousands).

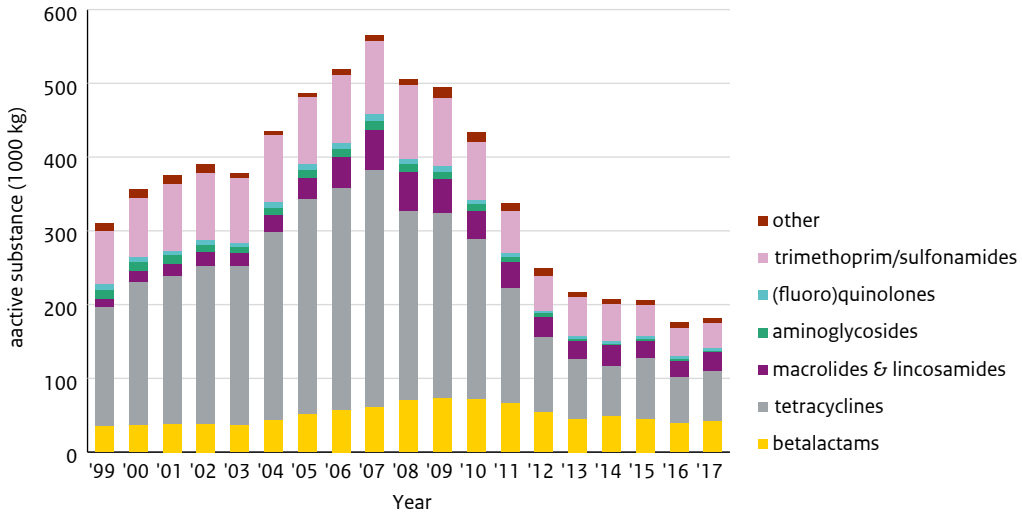
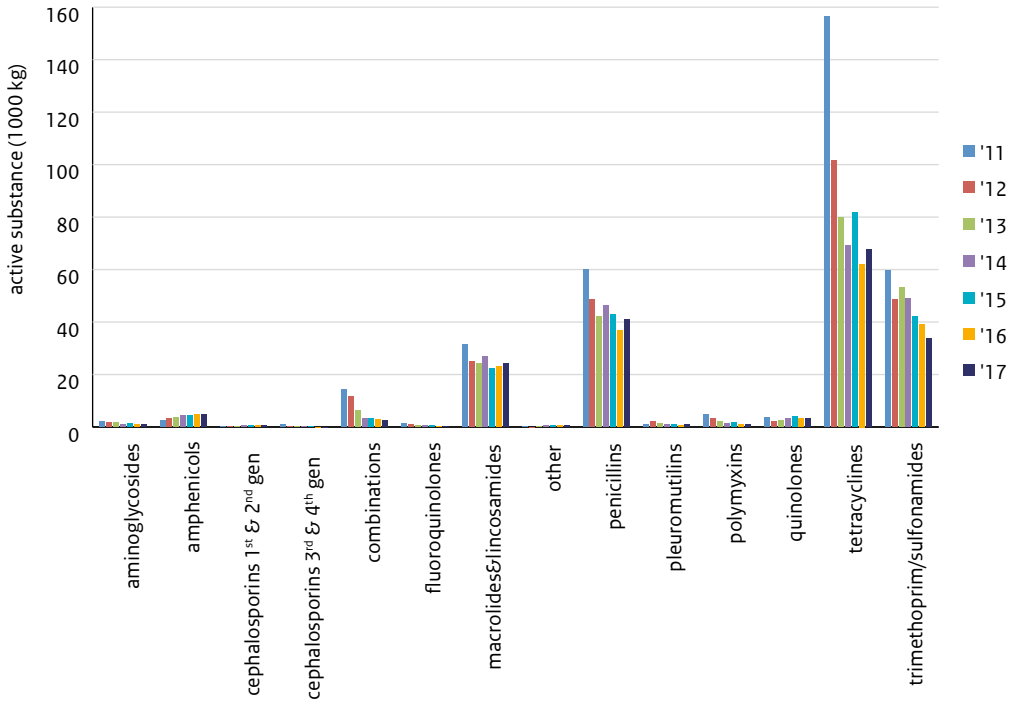


Table ABuse02 Antimicrobial veterinary medicinal product sales from 1999-2017 in kg (thousands) (FIDIN, 2018).

year	'99	'00	'01	'02	'03	'04	'05	'06	'07	'08	'09	'10	'11	'12	'13	'14	'15	'16	'17
betalactam antibiotics	35	36	38	38	36	43	51	57	61	70	73	71	66	54	45	48	45	39	42
tetracyclines	162	194	200	214	216	256	292	301	321	257	251	217	157	102	80	69	82	62	68
macrolids & lincosamides	10	15	17	19	17	23	28	42	55	52	46	39	34	26	25	28	23	23	25
aminoglycosides	13	12	11	10	9	9	11	11	12	11	10	8.6	7.3	5.8	3.4	1.8	2.7	2.1	1.9
(fluoro)quinolones	7	7	6	6	5	7	8	7	9	8	8	6.6	5.1	3.1	2.8	3.8	4.2	3.4	3.4
trimethoprim/ sulfonamides	72	80	92	92	88	91	91	93	99	100	92	78	58	48	53	49	42	39	34
other antibiotics	11	12	11	11	7	6	6	8	8	7	15	13	10	10	8.1	7.8	7.5	7.4	7.2
total sales	310	356	376	390	378	434	487	519	565	506	495	433	338	249	217	207	206	176	181

Figure ABuse02 Antimicrobial veterinary medicinal product sales by pharmaco-therapeutic class 2011-2017 in kg (thousands)



Tetracyclines

In contrast to 2016, total mass of tetracyclines sold increased with 9% in 2017, while the use decreased in all monitored sectors. This pattern also occured in 2015. The fraction of doxycycline increased to 49% of the total sales of tetracyclines (47% in 2016, 42% in 2015, 41% in 2014, 31% in 2013, 41% in 2012 and 34% in 2011).

Penicillins

Second place in mass again, penicillin sales increased 11% compared to 2016; the increase was limited to the broad spectrum aminopenicillins, sales of narrow spectrum penicillines decreased. As a result, the overall figures amount to 75% broad and 25% narrow spectrum penicillines of the mass sold respectively.

Trimethoprim/sulfonamides

The use of trimethoprim/sulfonamides decreased further in 2017, and due to the increase of penicillins, it ranks third in mass sold.

(Fluoro)quinolones

The sales of fluoroquinolones decreased with 82 kg (25%) in 2017. An overall reduction of 83% was realized in comparison with 2011. 78% of the sales are applied in the monitored sectors. The sales of quinolones increased in 2017, compared with 2011 an overall decrease of 14% was noticed, these substances are exclusively applied in the food producing sectors.

Cephalosporins

The sales of 1st and 2nd generation cephalosporins increased steeply in 2014 due to underreporting in previous years; two AVMP's for companion animals were reported for the first time. Sales of these VMPs were relatively stable over the period 2015 to 2017. The sales of 3rd and 4th generation cephalosporins halved in 2017 with 1 kg (from 2 kg). A reduction of 99.8% has been achieved since 2011. The availability of these product on the market has diminished steeply as a result from this decrease. For food producing animals no products are available anymore, in case of urgency AVMP's have to be imported.

Polymyxins

Colistin sales and use decreased in 2017. Compared to 2011 a reduction of 80% has been accomplished. 95% are oral VMP's, 5% are injectables combined with aminopenicillins.

2.2. Usage in pigs, veal calves, cattle, broilers and turkeys in the Netherlands

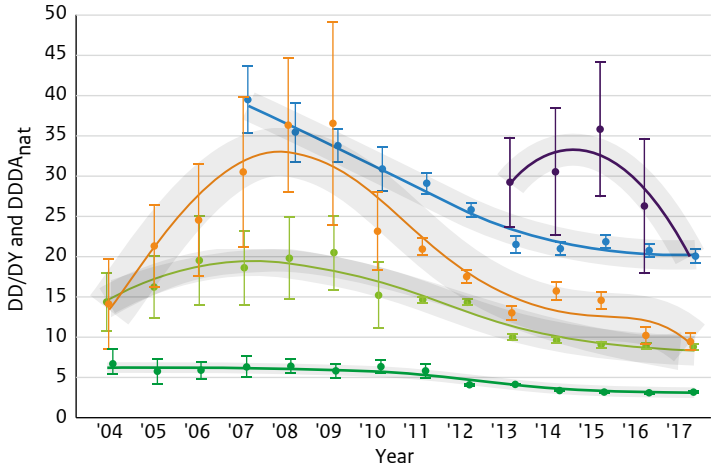
Starting in 2004, AVMP consumption data derived from veterinarian's invoices were collected in the Netherlands by Wageningen University for sentinel farms. These data were, in cooperation with Utrecht University, converted to the number of defined doses per animal year (DD/AY). The calculation method is similar to the method applied in human drug use. Applied antimicrobial veterinary medicinal products (AVMP's) are converted to treated animal mass*days by national conversion factors (determined by the nationally authorized dosages and pharmacokinetics of the drug to compensate for duration of action) and related to animal mass present on a farm. Results are calculated for a period of a year and expressed as the number of days an average animal is treated in that year on that particular farm.
The sentinel data (2004-2010) are weighted by farm related variables to obtain figures representative for the whole population of farms in a sector.

Since 2011, husbandry related consumption reports are prepared by the Netherlands Veterinary Medicines Institute (SDa) using consumption data from *all* farms in the largest sectors of food production animals: pigs, veal calves, broilers, cattle (since 2012) and turkeys (since 2013). In 2016 rabbits are also monitored but are not included in this report because of transition problems with data transfer. Since 2017 also antimicrobials use in poultry sectors additionally to broilers is made available. While the calculation method for treated body mass (numerator) is the same, totalized for all farms per sector, the denominator represents the whole sector, and this measure is referred to as Defined Daily Doses Animal ($DDDA_{NAT}$). Table ABuse03 shows the animal populations AVMP's consumption data are reported for in 2013 – 2017 (pigs, veal calves, cattle, broilers and turkeys).
Table ABuse04 gives animal weights applied in the calculation of the denominator. In Table ABuse05 the resulting $DDDA_{NAT}$ are shown. In most sectors (veal valves, pigs, broilers and turkeys) a reduction in consumption has been realized. In dairy cows and other cattle a small increase in consumption is noted. The trends in the number of defined daily dosages animal for the veal farming, sows/piglets farming, fattening pigs farming and broiler farming sectors as reported by LEI WUR-MARAN (years 2007-2010 as DD/AY) and by SDa (years 2011-2016 as $DDDA_{NAT}$) are depicted in Figure Abuse03, and specification of applied antimicrobial groups in the different sectors for 2013-2017 is presented in Figure Abuse04. $DDDA_{NAT}$ in 2011 is estimated by the 2011/2012 $DDDA_{F}$ ratio (weighted by average animal kgs present per farm). For veal calves all observations of 2007-2010 were recalculated with the average dosages of VMP's instead of maximum dosages as were applied for veal calves exclusively until 2013. For broilers the $DDDA_{NAT}$ in 2011 was estimated by the 2011/2012 treatment days ratio (treatment days are weighted by the number of animal days per farm) and the $DDDA_{NAT}$ in 2012 was estimated by treatment days adjusted by the 2013 treatment days/$DDDA_{NAT}$ ratio. From 2011 to 2017, CBS (Centraal Bureau voor de Statistiek, National Institute of Statistics) data for number of animals are used in the calculations for broilers, turkeys, veal calves and rabbits, and EUROSTAT data for pigs and dairy cattle. Confidence limits (CLs) are obtained from the corresponding CLs for $DDDA_{F}$ *in casu* weighted treatment days per year.

Table ABuse03 Weight per sector in kg (thousands) for DDD_{NAT} calculation.

Sector	2012	2013	2014	2015	2016	2017
pigs	710,688	710,802	704,937	706,025	686,638	690,093
veal calves	156,602	159,547	158,828	156,751	164,890	163,935
diary cows	924,600	958,200	966,000	1,030,200	1,076,400	999,000
other cattle	597,900	573,800	649,000	649,800	600,100	542,000
broilers	43,846	44,242	47,020	49,107	48,378	48,237
turkeys	4,961	5,046	4,763	5,178	4,572	4,023
rabbits	872	830	860	1,004	948	901

Figure ABuse03 Animal-defined daily dosages for turkeys (purple), veal calves (blue), broilers (orange), pigs (light green) and dairy cattle (dark green) farms as reported by LEI WUR-MARAN (years 2007-2010 as DD/AY) and by SDa (years 2011-2017 as $DDDA_{NAT}$) depicting point estimates (dots), 95% confidence limits (error bars), smoothed trend line (penalized spline) and 95% confidence limits for the spline (shaded area).



For benchmarking purposes, every farm in the Netherlands is periodically provided with the number of defined daily doses animal per year ($DDDA_F$) of the farm by the sector quality systems.
This consumption is calculated with a detailed denominator, to facilitate refined benchmarking.
Table ABuseo6 depicts the animal bodyweights applied in the calculation of the denominator of $DDDA_F$ by the SDa.

For more details, annual reports of the SDa can be consulted (http://autoriteitdiergeneesmiddelen.nl/en/publications).

Table ABuse04 Applied bodyweights for $DDDA_{NAT}$ calculation.

Species	Category	Standard Weight (kg)	
Veal Calves		172	
Pigs	Piglets (< 20 kg)	10	
	Sows	220	
	Fattening pigs	70.2	
	Other pigs	70	
Broilers		1	
Turkeys		6	
Cattle	Dairy cows	600	
	Other cows	500	
Rabbits	Dow+kits	8.4	
	Fattening rabbits	1.8	
	Other rabbits	3.4	

Figure ABuse04 Number of $DDDA_{NAT}$ per animal-year of antimicrobial veterinary medicinal products specified by pharmaco-therapeutic groups per animal sector over the years 2013-2017.

* *categorization in first, second and third choice antimicrobials based on Dutch WVAB guideline 2015*

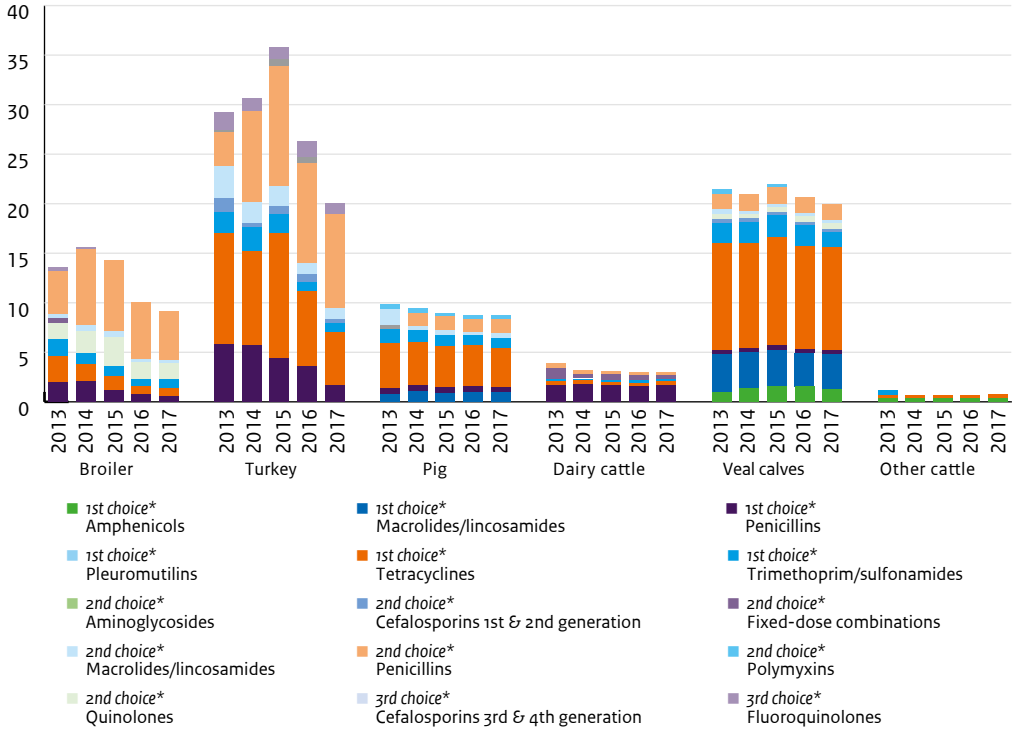


Table ABuse05 Trends in $DDDA_{NAT}$ in the Netherlands in livestock 2013 - 2017.

	Animalsector														
	Veal calfs*					Dairy cattle					Cattle				
Year	2013	2014	2015	2016	2017	2013	2014	2015	2016	2017	2013	2014	2015	2016	2017
Number of farms with prescriptions	2125	2061	1978	1928	1868	18005	17747	17737	17529	17121	13644	13359	12971	12548	12790
Pharmacotherapeutic group															
Aminoglycosides	0.53	0.34	0.19	0.23	0.23	0.00	0.00	0.01	0.01	0.01	0.02	0.01	0.01	0.01	0.01
Amphenicols	1.23	1.52	1.63	1.59	1.44	0.05	0.06	0.06	0.06	0.05	0.11	0.10	0.10	0.11	0.11
Cefalosporins 1st & 2nd generation	-	-	-	-	-	0.03	0.02	0.02	0.03	0.03	0.00	0.00	0.00	0.00	0.00
Cefalosporins 3rd & 4th generation	0.00	0.00	-	-	-	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Combinations	0.09	0.01	0.00	0.00	0.01	1.01	0.48	0.42	0.38	0.34	0.08	0.04	0.03	0.03	0.04
Fluoroquinolones	0.03	0.02	0.02	0.03	0.04	0.00	0.00	0.00	0.00	0.00					0.00
Macrolides/lincosamides	3.84	3.72	3.88	3.54	3.65	0.06	0.10	0.10	0.07	0.06	0.22	0.20	0.16	0.17	0.19
Other	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Penicillins	2.11	2.15	2.33	2.25	2.21	2.20	2.00	1.87	1.86	2.00	0.19	0.18	0.16	0.16	0.18
Pleuromutilins	-	-	-		-	-	-	-	-	-	-	-	-	-	-
Polymyxins	0.36	0.15	0.19	0.07	0.02	0.02	0.01	0.01	0.01	0.00	0.01	0.01	0.01	0.00	0.00
Quinolones	0.30	0.49	0.58	0.66	0.57	0.00	0.00	0.00	0.00	0.00	0.01	0.03	0.02	0.03	0.02
Tetracyclines	10.87	10.66	11.01	10.47	10.35	0.42	0.39	0.37	0.35	0.32	0.59	0.47	0.42	0.44	0.45
Trimethoprim/sulfonamides	2.14	2.08	2.22	2.05	1.61	0.22	0.24	0.25	0.24	0.24	0.16	0.11	0.10	0.10	0.09
Total	21.50	21.15	22.05	20.88	20.13	4.03	3.30	3.11	3.01	3.06	1.40	1.15	1.00	1.07	1.10

* *Population data derived from CBS (formerly from Eurostat)*

Table ABuse05 (continued) Trends in $DDDA_{nat}$ in the Netherlands in livestock.

	Animalsector														
	Pigs					Broilers					Turkeys				
Year	2013	2014	2015	2016	2017	2013	2014	2015	2016	2017	2014	2014	2015	2016	2017
Number of farms with prescriptions	6588	6072	5824	5462	5297	770	797	816	849	852	40	40	47	43	45
Pharmacotherapeutic group															
Aminoglycosides	-	0.01	0.01	0.01	0.01	0.03	0.03	0.02	0.01	0.03	1.24	0.40	0.71	0.69	0.05
Amphenicols	0.09	0.17	0.18	0.24	0.25	-	-	-	-	-	0.02	-	-	-	-
Cefalosporins 1st & 2nd generation	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Cefalosporins 3rd & 4th generation	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Combinations	0.10	0.05	0.04	0.03	0.02	0.37	0.08	0.11	0.05	0.01	-	-	-	-	-
Fluoroquinolones	0.00	0.00	-	0.00		0.24	0.18	0.07	0.07	0.05	1.76	1.29	1.20	1.60	1.06
Macrolides/lincosamides	1.02	1.09	1.04	1.08	1.13	0.31	0.35	0.48	0.25	0.20	3.55	2.12	1.98	1.18	1.30
Other	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Penicillins	2.17	2.05	1.93	1.97	1.96	6.34	9.96	8.44	6.48	5.58	9.34	14.89	16.61	13.75	11.01
Pleuromutilins	0.12	0.09	0.08	0.07	0.09	-	-	-	-	-	-	-	0.12	-	0.10
Polymyxins	0.44	0.34	0.38	0.28	0.26	0.08	0.05	0.06	0.04	0.03	0.18	0.08	0.63	0.61	-
Quinolones	0.03	0.05	0.03	0.02	0.03	1.65	2.22	2.86	1.51	1.72	0.23	0.02	0.10	0.01	0.26
Tetracyclines	4.58	4.34	4.15	4.07	4.05	2.52	1.77	1.49	1.01	0.95	11.19	9.58	12.57	7.63	5.51
Trimethoprim/sulfonamides	1.40	1.33	1.20	1.10	0.90	1.46	1.45	1.07	0.78	0.82	1.80	2.37	2.01	0.95	0.86
Total	9.97	9.52	9.05	8.87	8.70	13.01	15.76	14.59	10.19	9.40	29.31	30.74	35.94	26.42	20.16

* *Population data derived from CBS (formerly from Eurostat)*

Table ABuse06 Applied bodyweights for $DDDA_F$ calculation.

Species	Category	Specifications	Age	Standard weight (kg)
Calves	White veal		0-222 days	160
	Red veal startup		0-98 days	77.5
	Red veal fattening		98-256 days	232.5
	Red veal combination		0-256 days	205
Pigs	Sows/piglets	Sows (all female animals after 1st insemination) and boars		220
		Suckling piglets	0-25 days	4.5
		Gilts	7 months-1st insemination	135
	Weaned piglets		25-74 days	17.5
	Fattening pigs / gilts	Fattening pigs	74 days-5 months	70
		gilts	74 days-7 months	70
Broilers			0-42 days	1
Turkeys		male	0 - 20 weeks	10.5
		female	0 - 17 weeks	5.6
Cattle	Dairy cows /	female	>2 years	600
	Suckler cows /	female	1-2 years	440
	Bulls for meat /	female	56 days-1 year	235
	Rearing animals	female	<56 days	56.5
		male	>2 years	800
		male	1-2 years	628
		male	56 days-1 year	283
		male	<56 days	79
Rabbits	Dow+kits	combined weight		8.4
		Dow	> 3-5 months	
		Kits	0 - 4.5 weeks	
	Fattening rabbits		4.5 - 13 weeks	1.8
	Other rabbits	female	11 weeks - 5 months	3.4

2.3 Usage expressed in the number of international units DDDVET of the European Surveillance of Veterinary Antimicrobial Consumption in pigs, veal calves, cattle, broilers and turkeys in the Netherlands per animal-year

A comparison of the number of DDDA with the internationally established ESVAC DDD_{VET} was conducted for the 2016 and 2017 data, with the denominator of the $DDDA_{NAT}$ (live weight). This measure is included because it potentially facilitates international comparisons. The use is calculated excluding the locally administered AVMP's for mastitis and metritis, which are included in the Dutch system, but in the ESVAC system are only accounted for in the defined course dose (DCD_{VET}) calculation.

In general, both methods result in comparable consumption. In the Dutch system, AVMP's of a combination of active substances result in only one treatment day, while in the ESVAC approach application of such product results in one treatment day for every active substance. This difference in the group trimethoprim/sulfonamides affects all sectors, except turkeys. In turkeys a product with one sulfonamide is predominantly applied, with a much lower authorized dose in the Netherlands than the average dose in Europe. Table Abuse07 depicts the results of antimicrobial consumption in European DDD_{VET} per (live weight) animal-year.

In contrast to the SDa $DDDA_{NAT}$ calculations, DDD_{VET} results decreased for all sectors, even in dairy cows and other cattle. In dairy cows this could imply that the increase was caused by mastitis and dry cow treatments, being excluded from calculation in de ESVAC method. In veal calves this explanation is less obvious.

Conclusion

Maximal transparency has been created since 2011 through monitoring antibiotics use by veterinarians and farmers. The unexpected increase in sales of antimicrobial VMP's in the Netherlands in 2017 may be the result of an adjustment or compensation for the relatively low 2016 sales, which is not supported by the use monitoring data. The calculation of consumption is based on national conversion factors (DDDAs) of authorized drugs.

The use of antibiotics of critical importance to human health care (especially cephalosporins of 3rd and 4th generation) is reduced to an absolute minimum, even in the unmonitored sectors. Import of these VMP's from other EU member states is not monitored in sales data, but if used in the monitored animal sectors, veterinarians are obliged to report these VMP's.

Table ABuse07 number of DDDAVET/animal year in monitored sectors 2016-2017.

	Broilers		Turkeys		Pigs		Dairy cattle (excluding intramammary and intrauterine administrations)		Veal calves		Other cattle	
	2016	2017	2016	2017	2016	2017	2016	2017	2016	2017	2016	2017
	#DDD$_{VET}$	#DDD$_{VET}$	#DDD$_{VET}$	#DDDA	#DDD$_{VET}$	#DDD$_{VET}$	#DDD$_{VET}$	#DDD$_{VET}$	#DDD$_{VET}$	#DDDA	#DDD$_{VET}$	#DDD$_{VET}$
First choice*	4.02	3.71	16.12	11.37	6.91	6.62	0.95	0.92	19.51	18.52	0.95	0.95
% 1st choice of total	34.84%	34.36%	57.72%	49.48%	79.13%	77.72%	90.33%	89.76%	78.93%	87.61%	81.28%	86.12%
Amphenicols	-	-	-	-	0.18	0.19	0.04	0.04	1.22	1.11	0.09	0.08
Macrolides/lincosamides	-	-	-	-	0.81	0.85	0.03	0.03	3.81	3.94	0.17	0.19
Penicillins	0.68	0.58	3.64	1.61	0.57	0.54	0.15	0.15	0.26	0.26	0.05	0.05
Pleuromutilins	-	-	-	0.14	0.07	0.10	-	-	-	-	-	-
Tetracyclines	1.32	1.27	10.71	9.20	3.46	3.42	0.24	0.22	10.88	10.61	0.47	0.48
Trimethoprim/sulfonamides	1.78	1.86	0.49	0.42	1.81	1.51	0.47	0.48	3.34	2.61	0.17	0.15
Second choice*	7.47	7.03	10.21	10.54	1.82	1.90	0.10	0.10	5.18	2.59	0.22	0.15
% 2nd choice of total	64.55%	65.15%	36.55%	45.89%	20.87%	22.28%	9.34%	9.97%	20.97%	12.23%	18.68%	13.81%
Aminoglycosides	0.00	0.03	0.20	0.01	0.00	0.00	0.01	0.01	0.09	0.09	0.01	0.01
Cefalosporins 1st & 2nd generation	-	-	-	-	-	-	-	-	-	-	-	-
Combinations	1.08	0.02	0.01	-	0.02	0.03	0.00	0.04	0.85	0.01	0.04	0.03
Macrolides/lincosamides	0.33	0.19	1.28	1.40	0.08	0.53	0.04	0.01	0.00	0.14	0.03	0.01
Penicillins	-	5.53	-	8.95	0.41	1.01	0.01	0.05	0.12	1.59	0.01	0.07
Polymyxins	6.28	0.02	9.56	0.00	0.97	0.31	0.04	0.00	4.05	0.02	0.13	0.00
Quinolones	0.03	1.23	0.44	0.19	0.34	0.02	0.01	0.00	0.07	0.74	0.01	0.03
Third choice*	0.07	0.05	1.60	1.06	0.00	0.00	0.00	0.00	0.02	0.03	0.00	0.00
% 3rd choice of total	0.61%	0.49%	5.73%	4.63%	0.00%	0.00%	0.33%	0.27%	0.10%	0.16%	0.03%	0.07%
Cefalosporins 3rd & 4th generation	-	-	-	-	-	-	0.00	0.00	-	-	0.00	-
Fluoroquinolones	0.07	0.05	1.60	1.06	0.00	0.00	0.00	0.00	0.02	0.03	0.00	0.00
Total	11.57	10.78	27.93	22.98	8.73	8.52	1.05	1.03	24.72	21.15	1.17	1.10

* *Categorization in first, second and third choice antimicrobials based on Dutch WVAB guideline 2015.*

3 Resistance data

This chapter describes susceptibility test results as determined in 2017 for the food-borne pathogens *Salmonella enterica* enterica, *Campylobacter* spp. and *Escherichia coli* O157, and the commensal organism *E. coli*. Epidemiological cut-off values (www.eucast.org) were used for the interpretation of minimum inhibitory concentrations (MIC). Epidemiological cut-off (ECOFF) values are in most cases lower than clinical breakpoints, and therefore, depending on the antibiotic, non-wild type susceptible isolates (isolates displaying MICs above the ECOFFs) cannot automatically be classified as clinically resistant. For the purpose of this report we designated all non-wild-type susceptible isolates as "resistant", and specified this per antibiotic if necessary.

3.1 Food-borne pathogens

3.1.1 Salmonella

In this chapter, resistance percentages of *Salmonella* isolates are presented. These isolates were sampled from humans suffering from clinical enteral infections/acute gastroenteritis, food-producing animals and food products from animals, as potential sources for distribution to humans via the food chain, and animal feeds as potential source for food-producing animals.

Highlights

1. In 2017 *S.* Enteritidis (25.6%) followed by *S.* Typhimurium (15.9%) together with the monophasic variant of Typhimurium: *S.* enterica subspecies enterica 1,4,[5],12:i:- (15.7%), were most frequently isolated from humans suffering from salmonellosis.
2. In pigs, the monophasic variant of *S.* Typhimurium dominated. In cattle, *S.* Typhimurium and *S.* Dublin were most commonly isolated.
3. In poultry (including poultry products and broilers), the number of *S.* Paratyphi B var. Java was equal to 2016. The most isolated serovar in poultry meat in 2017 was *S.* Heidelberg.
4. The highest proportions of resistance were observed in the *S.* Heidelberg, monophasic *S.* Typhimurium and in *S.* Kentucky, and to a lesser extent in *S.* Typhimurium.
5. Ciprofloxacin resistance was most common amongst isolates from humans and poultry. Predominant serovars were *S.* Kentucky (81.3% resistant), *S.* Infantis (26.2%) and Enteritidis (21.5%).
6. In 2017, the proportions cefotaxime resistant (MIC > 0.5 mg/L) ESBL suspected *Salmonella* isolates was 1.8%, among seven different serovars, isolated from human samples. Cefotaxime resistance was detected in 67.6% of the *Salmonella* isolates (predominantly S. Heidelberg) obtained from imported poultry products. No cefotaxime resistant isolates were found in fresh retail meat.
7. In 2017 no carbapenemase producing *Salmonella* were found.

Salmonella serovar prevalence

In the Netherlands, an extensive surveillance of *Salmonella* is carried out by the Dutch National Institute of Public Health and the Environment (RIVM), the EU reference laboratory (EU-RL) for *Salmonella* (EC 882/2004). A summary of the serotyping results of *Salmonella* isolated from humans and farm animals (pigs, cattle and poultry) is presented in Table S01.

From all human *Salmonella* isolates sent to the RIVM by regional public health and other clinical laboratories a selection of 1222 isolates was sent to WBVR for susceptibility testing. These strains were the first isolates recovered from patients with salmonellosis. Also, 475 isolates from other sources were tested consisting of: isolates from pigs (N = 50) and cattle (N = 40) sent to the RIVM by the Animal Health Service in Deventer from a diversity of surveillance programs and clinical *Salmonella* infections in animals. The isolates from broilers (N = 58) and layers and reproduction poultry (N = 8) were mainly nonclinical *Salmonella* isolates derived from a diversity of monitoring programs on farms, slaughterhouses and at retail. Isolates from a diversity of other sources (N = 319 from animal feed and food products; other animals from animal husbandry (e.g. sheep, goats) have also been serotyped and tested. In addition, NVWA tested 143 *Salmonella* isolates obtained from raw meats (mainly poultry), spices, herbs and seafood. The results of these isolates were not included in Tables S02, S03, S04 and S05, but are shown in Table S06.

In 2017, Enteritidis 02-10(11)-07-03-02 outbreak in humans was a continuation of the Polish egg outbreak in 2016; Monophasic Typhimurium 03-12-09-00-211 outbreak was at the German border and supposedly related to “junkfood” involving predominantly adolescents; Bovismorbificans outbreak related to the consumption of uncooked ham products; Kentucky outbreak took place in a nursery home; Newport , Agbeni and Infantis elevations could not be traced to a source.

As in previous years, *S.* Enteritidis and *S.* Typhimurium were the most frequently isolated serovars from human clinical infections. In 2017, the most frequently isolated from humans suffering from

salmonellosis were S. Enteritidis (25.6%), followed by S. Typhimurium (15.9%) together with the monophasic variant of Typhimurium (*S. enterica subspecies enterica* 1,4,[5],12:i:-) (15.7%).
S. Typhimurium and its monophasic variant were mainly associated with pigs and cattle, but were also found in poultry. S. Enteritidis was mainly isolated from poultry, broilers and layers (Table S01).
In pigs, the most isolated serovar was S. Typhimurium and especially its monophasic variant. In cattle, S. Typhimurium and S. Dublin were most commonly isolated. In poultry many different serovars were found. In 2017, the most isolated serovar was S. Heidelberg (27.2%) all from imported poultry meat or meat preparations, followed by S. Enteritidis (12.9%), which was the predominant serovar in 2016.
The presence of S. Paratyphi B var. Java (S. Java) and S. Infantis was approximately the same as in 2016 (9.6% and 6.6% respectively).
Reported travel, on average 10%, contributed up to 34% of the cases of human salmonellosis over the years 2014-2017, but differed per serovar. Relative high contributions of travel (≥30%) were noted for the serovars Kentucky, Typhi/Paratyphi A,B,C, Schwarzengrund, Stanley, Virchow and Corvallis.
It should be noted that the contribution of travel as presented in Table S01 is only indicative of the true contribution, because travel is underreported by an estimated factor of about two.

Resistance proportions

The in November 2013 implemented EU legislation on monitoring and reporting of antimicrobial resistance in zoonotic and commensal bacteria (2013/652/EU), includes susceptibility testing of mandatory panels of antimicrobials. For the monitoring of *Salmonella* three antibiotic compounds (azithromycin, meropenem and tigecycline) used in human medicine, but not in veterinary practice, have been added to the panel and three antimicrobials of less importance for treatment of human infections (florfenicol, kanamycin and streptomycin) have been deleted since the implementation (Table S02). Tigecycline is structurally related to tetracyclines, but has a broader spectrum of activity. Azithromycin is a potent macrolide and in human medicine often used instead of erythromycin for treatment of infections by Gram-positive bacteria, due to the effectiveness of a once-daily administration during a few days. Given its activity against Enterobacteriaceae and its favourable pharmacokinetics, it is also used for typhoidal *Salmonella* cases for which *in vivo* efficacy has been demonstrated. Meropenem belongs to the carbapenems, which are last resort antimicrobials that are used to treat infections with multi-drug resistant bacteria. Colistin has been used widespread in veterinary medicine for prevention and treatment of diarrhoeal diseases in livestock. In human medicine, colistin can be used for treatment of human infections with multidrug-resistant carbapenemase producing bacteria. For this reason, the use of colistin in veterinary medicine has been reduced in Dutch livestock. Moreover, the recent finding of a plasmid mediated colistin resistance gene (*mcr-1*) resulted in even more attention for this compound. Like in former years, colistin resistance was not reported in *Salmonella* in 2017. That is because an epidemiological cut-off value that can be applied for all *Salmonella* serovars is lacking for colistin, which makes the results difficult to interpret. Using the former ECOFF of 2 mg/L (which is also the clinical breakpoint) resistance rates would have been highly influenced by differences in natural susceptibility (wildtype strains of S. Enteritidis and S. Dublin are less susceptible to colistin). As a result, colistin resistance would have been over-reported in *Salmonella*.
All *Salmonella* with elevated colistin MIC-values (colistin MIC > 2 mg/L for most *Salmonella* and MIC > 4 mg/L for Dublin and Enteritidis) were screened with PCR for the presence of *mcr*-genes (see section 4.3).

Table S01 Most prevalent *Salmonella* serotypes isolated in 2016 and 2017 from humans, pigs (including pork), cattle (including beef), layers (including reproduction animals and eggs) poultry, broilers (including poultry products) and the % travel related human infections.

		Travel related	Humans		Pigs		Cattle	
		2014-2017	2016	2017	2016	2017	2016	2017
N Total			1529	1242	63	163	58	80
N tested	Tested		1473	1222	52	66	49	55
Enteritidis	841	12%	438	318			3	1
Typhimurium	601	4%	260	197	14	56	16	28
Typhimurium (monofasisch)	530	4%	229	195	33	86	14	10
Infantis	182	10%	37	44	1	1		
Paratyphi B. var. Java	86	24%	34	20			1	1
Kentucky	85	30%	36	40				
Dublin	82	3%	28	7	1	1	17	27
Heidelberg	82	10%	5	1				
Bovismorbificans	69	5%	42	28	1	2		1
Typhi/Paratyphi A,B,C	62	34%	31	24				
Derby	60	7%	20	12	5	5		
Brandenburg	58	4%	11	7	4	2	3	1
Newport	57	21%	23	24				1
Montevideo	53	21%	4	9				3
Livingstone	49	4%	5	4	1	2		
Agona	45	24%	13	10				1
Schwarzengrund	44	30%	9	6				
Napoli	42	9%	31	10				
Senftenberg	42	16%	5	1				
Kedougou	41	n.a.						
Chester	35	16%	16	16				
Mbandaka	33	28%	6	2				
Anatum	32	28%	1	2				
Stanley	29	31%	14	13				
Give	28	18%	4	6				
Thompson	27	3%	9	7				
Virchow	24	30%	9	11				
Saintpaul	23	25%	13	9				
Corvallis	22	34%	9	9				
Goldcoast	21	3%	8	6	1	1	2	1
Braenderup	19	24%	12	7				
Tennessee	18	3%	1					
Weltevreden	18	31%	10	6				
Javiana	17	19%	6	12				
Rissen	17	19%	5	3	2			
Panama	17	8%	4	6		2		
Agbeni	16	0%	1	16				
Bredeney	15	22%	4	3				
Ohio	15	13%	1	3				
Poona	15	26%	6	7				
Hadar	12	27%	5	5				

Table S01 (continued) Most prevalent *Salmonella* serotypes isolated in 2016 and 2017 from humans, pigs (including pork), cattle (including beef), layers (including reproduction animals and eggs) poultry, broilers (including poultry products) and the % travel related human infections.

	Poultry		Broiler		Layer		Other	
	2016	2017	2016	2017	2016	2017	2016	2017
N Total	318	272	98	160	112	24	1028	926
N tested	199	197	76	139	49	8	354	337
Enteritidis	183	35	39	6	85	5	33	47
Typhimurium	9	19	1	7	2	4	38	107
Typhimurium (monofasisch)	24	21	13	5	3	7	41	78
Infantis	32	18	20	10	2		74	33
Paratyphi B. var. Java	23	26	9	22	2		16	7
Kentucky							6	8
Dublin		1					7	1
Heidelberg	2	74	2	73			2	1
Bovismorbificans		2					1	
Typhi/Paratyphi A,B,C								
Derby		1					35	15
Brandenburg	1	5	1	5			42	22
Newport	1	1	1	1			3	2
Montevideo	2	2			1	1	31	24
Livingstone							146	109
Agona		2		1			18	14
Schwarzengrund	2	8	2	8			11	17
Napoli							3	3
Senftenberg		2					29	22
Kedougou		6		2			19	65
Chester							7	2
Mbandaka	2	1		1	2		16	16
Anatum	2	3		3	1		68	15
Stanley							3	4
Give	3		1				4	21
Thompson		11				4	4	8
Virchow	2	2	2	1			3	3
Saintpaul							4	5
Corvallis	2		1				2	2
Goldcoast							1	4
Braenderup							2	2
Tennessee	1		1				43	7
Weltevreden							6	1
Javiana							1	1
Rissen							8	3
Panama	2				2		4	2
Agbeni								
Bredeney		1				1	18	2
Ohio							5	13
Poona							1	3
Hadar	1						6	5

Table S01 (continued) Most prevalent *Salmonella* serotypes isolated in 2016 and 2017 from humans, pigs (including pork), cattle (including beef), layers (including reproduction animals and eggs) poultry, broilers (including poultry products) and the % travel related human infections.

	Travel related		Humans		Pigs		Cattle	
	2014-2017		2016	2017	2016	2017	2016	2017
N Total			1529	1242	63	163	58	80
N tested	Tested		1473	1222	52	66	49	55
Oranienburg	12	21%	5	8				
Bareilly	11	21%	6	4				
Kottbus	10	24%	5	4				1
Muenchen	10	17%	2	6		1	1	
Cerro	8	25%	1					
Goettingen	8	0%	3	1				1
Jerusalem	8	n.a.						
London	8	6%	1	3		1		
Indiana	7	10%	3					
Mikawasima	6	0%	4	2				
OVERIGE	352	18%	94	108		3	1	3

MIC-distributions and resistance percentages of 1697 *Salmonella's* from different sources tested for susceptibility in 2017 are presented in Table S02. The resistance rates were approximately at the same level as in 2016. Highest proportions of resistance were again observed for sulfamethoxazole, tetracycline, ampicillin, and to a lesser extent for ciprofloxacin, nalidixic acid, chloramphenicol and trimethoprim. The proportions of resistance to ciprofloxacin and cefotaxime/ceftazidime seem to fluctuate a little since 2013. Resistance to the carbapenem antibiotic meropenem was not detected, indicating that carbapenemase producers were not present in the tested isolates (see also chapter 4.2). Like in 2015 and 2016, low proportions of resistance were found for tigecycline (1.3%) and azithromycin (1.0%), almost exclusively in human isolates.

Table S03 shows resistance percentages for the twelve most prevalent serovars isolated in the Netherlands in 2017. Resistance profiles varied considerably among serovars. High resistance proportions were observed in *S.* Heidelberg, monophasic *S.* Typhimurium and in *S.* Kentucky (64.6-81.3%), and to a lesser extent in *S.* Typhimurium.
Most serovars have acquired resistance against more than one antimicrobial. Again, the most common pattern was resistance to ampicillin, sulfamethoxazole and tetracycline (ASuT).

Quinolone resistance

The class of fluoroquinolones is widely regarded as the treatment of choice for severe salmonellosis in adults. Currently, EUCAST recommends a clinical breakpoint of 0.06 mg/L for *Salmonella enterica*, based on clinical evidence that there is a poor therapeutic response in systemic infections caused by *Salmonella* spp. with low-level ciprofloxacin resistance (MIC >0.06 mg/L) (www.eucast.org). Using the EUCAST recommended epidemiological cut off value of 0.06 mg/L as breakpoint, 13.8% of *Salmonella* isolates (N =234/1697) demonstrated an acquired resistance phenotype for ciprofloxacin (Table S02).
The dominant serovars of ciprofloxacin resistant isolates were *S.* Heidelberg (100%), *S.* Kentucky (81%) from humans, *S.* Infantis (26%) from broilers, and *S.* Enteritidis (22%) from both humans and broilers.

Table S01 (continued) Most prevalent *Salmonella* serotypes isolated in 2016 and 2017 from humans, pigs (including pork), cattle (including beef), layers (including reproduction animals and eggs) poultry, broilers (including poultry products) and the % travel related human infections.

	Poultry		Broiler		Layer		Other	
	2016	2017	2016	2017	2016	2017	2016	2017
N Total	318	272	98	160	112	24	1028	926
N tested	199	197	76	139	49	8	354	337
Oranienburg							7	3
Bareilly								
Kottbus								
Muenchen							2	2
Cerro							5	5
Goettingen								
Jerusalem	4	8		5	4		2	1
London							5	2
Indiana	2				2		3	1
Mikawasima							1	
OVERIGE	18	23	5	10	6	2	242	218

In meat (Table S06) the proportion of isolates resistant to ciprofloxacin was very high (89%). The majority of these isolates were obtained from chicken meat (both from imported meat and fresh retail meat). In chicken meat *S.* Heidelberg (N=72) (all from imported meat) was the most predominant isolate followed by *S.* Schwarzengrund (N = 8) and *S.* Infantis (N = 7). The high proportion of resistance to fluoroquinolones in poultry meat reflects the frequent usage of fluoroquinolones in the international poultry production chain.

ESBL's in *Salmonella*

The emergence of multidrug resistant *Salmonella* strains with resistance to fluoroquinolones and third-generation cephalosporins is a serious development, which results in severe limitations for effective treatment of human infections (WHO, factsheet 139, 2005). The total number of cefotaxime resistant (MIC > 0.5 mg/L) ESBL suspected *Salmonella* isolates in 2017 was 31/1697 (1.8%), among seven different serovars, all isolated from human samples: predominantly *S.* Kentucky (N = 18) and, *S.* Typhimurium (N = 8). The other serovars were *S.* Infantis (N = 2), monophasic *S.* Typhimurium (N = 1), S. Agona (N=1) and *S.* Virchow (N=1).
In isolates from imported meat samples (from outside EU) the proportion of cefotaxime resistance in imported chicken meat was high with 67.6% (Table S06). The serovars were *S.* Heidelberg (N = 65), *S.* Minnesota (N = 2) and *S.* Schwarzengrund (N = 1). No cefotaxime resistance was detected in samples from fresh retail chicken meat, or other fresh meat products.

Table S02 MIC distribution (in %) and resistance percentages (R%) for all *Salmonella*'s (N=1697) tested for antibiotic susceptibility during 2017.

Salmonella	MIC (%) distribution mg/L																	R%	95% CI
N = 1697	0.015	0.03	0.06	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	512	1024	2048	
Ampicillin							37.9	32.2	1.8	0.1			0.1	27.9					27.9	25.8 - 30.1
Cefotaxime					97.1	1.0	0.1			1.8									1.8	1.2 - 2.6
Ceftazidime						95.1	3.2	0.2	0.5	0.5	0.5								1.4	0.9 - 2.1
Gentamicin						85.9	9.8	0.9	0.4	0.1	1.2	0.9	0.8						3.4	2.6 - 4.4
Tetracycline								71.9	2.5	0.4	0.1	0.9	2.2	22.0					25.2	23.1 - 27.3
Sulfamethoxazole										45.1	24.6	3.5	0.5	0.1			0.1	26.2	26.2	24.1 - 28.4
Trimethoprim					68.4	23.0	1.0	0.3	0.1				7.3						7.4	6.2 - 8.7
Ciprofloxacin	15.1	69.0	2.1	0.8	4.7	4.0	1.4	0.3		0.6	1.9								13.8	12.2 - 15.6
Nalidixic acid									79.8	6.4	2.1	1.7	0.1	0.2	9.7				11.7	10.2 - 13.3
Chloramphenicol										87.5	6.2	0.4	0.2	0.6	5.2				6.3	5.2 - 7.6
Azitromycin*								0.1	24.3	69.5	5.2	0.3	0.2	0.5					1.0	0.6 - 1.6
Colistin**							79.4	13.1	7.1	0.4									-	-
Meropenem		86.3	13.6	0.1															0.0	0 - 0.2
Tigecyclin					55.2	37.5	6.1	1.2	0.1										1.3	0.8 - 2.0

The white areas indicate the dilution range tested for each antimicrobial agent. Values above this range indicate MIC values > the highest concentration in the range. Values at the lowest concentration tested indicate MIC-values ≤ the lowest concentration in the range. Vertical bars indicate the epidemiological cut-off values (ECOFF), used as breakpoints. If available, dashed bars indicate the clinical breakpoints. For ampicillin, ciprofloxacin and chloramphenicol the ECOFF and clinical breakpoints are identical.

* *tentative set ECOFF during the EURL AMR WP meeting on 25 April 2015 in Lyngby (DK).*

** *Because of differences in natural susceptibility for colistin between serovars there is no general Salmonella ECOFF available for colistin. For this reason the percentage of resistance is not depicted*

Table S03 Resistance (%) of the twelve most prevalent *Salmonella* serovars isolated in the Netherlands in 2017 (N tested).

	Enteritidis (326)	Typhimurium (272)	1,4,[5],12:i:- (235)	Infantis (65)	Kentucky (48)	Bovismorbificans (34)	Kedougou (32)	Montevideo (31)	Dublin (26)	Livingstone (26)	Newport (25)	Paratyphi B var Java (25)
Ampicillin	8.6	60.3	90.6	4.6	79.2	0.0	0.0	0.0	11.5	0.0	0.0	0.0
Cefotaxime	0.0	2.9	0.4	4.6	37.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Ceftazidime	0.0	0.7	0.4	1.5	37.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Gentamicin	0.3	5.1	2.1	1.5	64.6	0.0	0.0	0.0	7.7	0.0	0.0	0.0
Tetracycline	2.1	43.0	88.9	20.0	70.8	2.9	0.0	0.0	15.4	0.0	12.0	0.0
Sulfamethoxazole	2.1	46.7	88.9	26.2	68.8	2.9	0.0	3.2	19.2	7.7	12.0	8.0
Trimethoprim	0.0	20.2	6.4	15.4	6.3	0.0	0.0	3.2	11.5	7.7	12.0	16.0
Ciprofloxacin	21.5	6.6	4.3	26.2	81.3	0.0	0.0	6.5	3.8	0.0	12.0	8.0
Nalidixic acid	21.2	2.2	2.6	26.2	81.3	0.0	0.0	3.2	3.8	0.0	4.0	8.0
Chloramphenicol	0.0	22.8	8.9	4.6	6.3	0.0	0.0	0.0	15.4	0.0	8.0	0.0
Azithromycin	0.6	0.7	3.0	0.0	2.1	0.0	0.0	0.0	7.7	0.0	0.0	0.0
Meropenem	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Tigecycline	0.0	3.3	0.4	6.2	12.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0

S. Typhimurium

S. Typhimurium represented 15.9% (197/1242) of all human *Salmonella* isolates as characterized by RIVM in 2017 (Table S01). This is less than in 2015 and 2016 (19.4% and 17.0% respectively), and approximately the same as in 2014 (16.2%). *S.* Typhimurium is a common serotype in animals. If the monophasic Typhimurium variant is included, *S.* Typhimurium may be regarded as the most dominant serotype in humans and food-producing animals like pigs and cattle.

Table S04 shows that resistance in *S.* Typhimurium was very high for ampicillin, tetracycline and sulfamethoxazole, for chloramphenicol in cattle isolates and also for trimethoprim in pig isolates and isolates from other sources (including broilers, sheep, goats, food and feed). Resistance to chloramphenicol was also found in isolates from humans, pigs and other sources, at a somewhat lower level. About 20% of the *S.* Typhimurium isolates exhibited the resistance profile Ampicillin-Chloramphenicol-Sulfamethoxazole-Tetracycline (ACSuT). Although streptomycin is not tested anymore, these figures indicate that the proportion of the penta-resistant phenotype (ACSuST) based on the chromosomal *Salmonella* Genomic Island 1, is similar to the proportion in previous years (except for 2015). Resistance to the clinically important drug cefotaxime was only detected in human isolates at a low level (3.9%). Resistance to fluoroquinolones was present in isolates from humans (7.8%), but was much less frequently found than in 2016 (19.2%). In cattle and pig isolates no resistance to fluoroquinolones was measured in 2017. After an increased finding of fluoroquinolone resistance in

S. Typhimurium isolates in 2016, proportions of resistance in 2017 were at the same level as in 2015. Borderline resistance to tigecycline was rarely observed in human isolates (N = 3), goats (N=3), sheep (N = 1) and pigs (N = 1). These isolates all exhibit slightly elevated MIC-values caused by an unknown resistance mechanism (if any).

Table S04 Resistance percentages of S. Typhimurium (N tested) isolated from humans, cattle, pigs and other sources in 2017.

	S. Typhimurium (272)[a]			
	Humans (206)	Cattle (16)	Pigs (18)	Other sources (33)[b]
Ampicillin	54.6	62.5	83.3	81.8
Cefotaxime	3.9	0.0	0.0	0.0
Ceftazidime	1.0	0.0	0.0	0.0
Gentamicin	3.9	37.5	0.0	0.0
Tetracycline	33.7	81.3	61.1	72.7
Sulfamethoxazole	38.0	81.3	61.1	75.8
Trimethoprim	13.7	25.0	38.9	48.5
Ciprofloxacin	7.8	0.0	0.0	6.1
Nalidixic acid	2.4	0.0	0.0	3.0
Chloramphenicol	21.0	56.3	16.7	21.2
Azithromycin	1.0	0.0	0.0	0.0
Meropenem	0.0	0.0	0.0	0.0
Tigecycline	1.5	0.0	5.6	15.2

a. monophasic variants (1,4,[5],12:i:-) are excluded.

b. including broilers, sheep, goats, food and feed products.

Resistance proportions in S. Typhimurium isolates from human samples showed an increasing tendency until 2010, after which resistance showed a tendency to decrease until 2015, with a slight increase for some antimicrobials in 2014, and an increase for most antimicrobials in 2016. Resistance proportions for cefotaxime and gentamicin, although being at low level, showed an increasing tendency as from 2011, and fluctuated from 2014 to 2016 (Figure S01). In 2017, resistance proportions for most antimicrobials were a bit lower, compared to 2016, except for ampicillin, trimethoprim, gentamicin and cefotaxime.

Resistance proportions in S. Typhimurium isolates from animal samples (cattle and pigs shown in figure S01) vary considerably over the years. This seemed to decrease from 2013, but an increase was seen in 2016. In 2017, resistance for almost all antimicrobials decreased in the isolates from pigs and increased in the isolates from cattle. However, these figures should be interpreted with care, because of the relatively small number of isolates per year.

S. Enteritidis

In the Netherlands, human infections caused by S. Enteritidis are mainly related to the consumption of contaminated eggs and, to a lesser extent, of poultry meat products and travel abroad. MLVA-typing is used to differentiate between types isolated from Dutch broilers and humans. The four dominant MLVA-types (03-10-05-04-01, 03-11-05-04-01, 03-09-05-04-01 and 02-10-07-03-02) were found in isolates from humans and broilers and were similar to the most predominant MLVA types in 2013 to 2016. In 2017, the most predominant (N = 77) S. Enteritidis again was MLVA type (02-09-07-03-02) part of the outbreak associated with the consumption of Polish eggs in 2016.

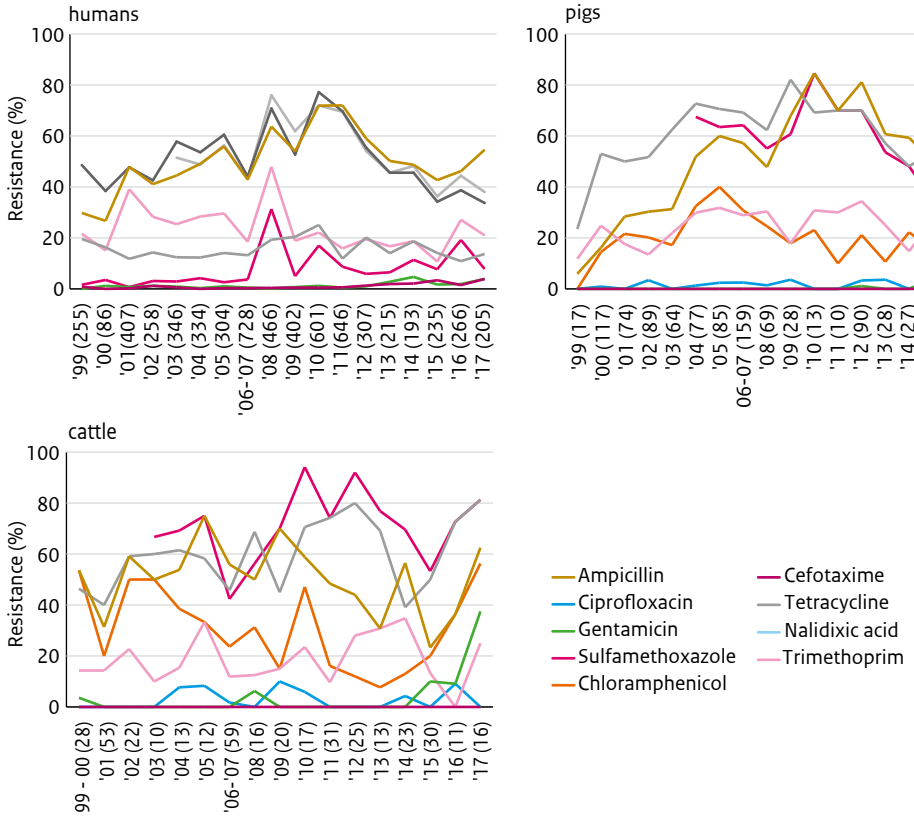
Compared to many other *Salmonella* serovars, resistance in S. Enteritidis is relatively low (Table S03). Table S05 presents resistance proportions in S. Enteritidis isolates from human samples and broilers. In 2017 no isolates from laying hens were tested, so we cannot compare with previous years.

The resistance percentage for fluoroquinolones in human isolates was 22.8%. For ampicillin a resistance rate of 9.1% was found. For all other antimicrobials resistance proportions of human S. Enteritidis isolates was very low or not detected. All isolates (N = 18) from broilers were fully susceptible. The trends in resistance of S. Enteritidis over the years in human isolates are summarized in Figure S02. Resistance in human isolates for chloramphenicol and ciprofloxacin increased, compared to 2016. In general, resistance proportions in human isolates seem to be very stable over years, with an increasing trend for ciprofloxacin resistance since 2010.

Table S05 Resistance percentages of S. Enteritidis (N tested) isolated from humans and broilers in 2017.

	S. Enteritidis (325)	
	Humans (307)	Broilers (18)
Ampicillin	9.1	0.0
Cefotaxime	0.0	0.0
Ceftazidime	0.0	0.0
Gentamicin	0.3	0.0
Tetracycline	2.3	0.0
Sulfamethoxazole	2.0	0.0
Trimethoprim	0.0	0.0
Ciprofloxacin	22.8	0.0
Nalidixic acid	22.5	0.0
Chloramphenicol	0.0	0.0
Azithromycin	0.7	0.0
Meropenem	0.0	0.0
Tigecycline	0.0	0.0

Figure S01 Trends in resistance (%) of S. Typhimurium isolated from humans and food-animals in 1999 - 2017.



S. Paratyphi B var. Java (*S.* Java)

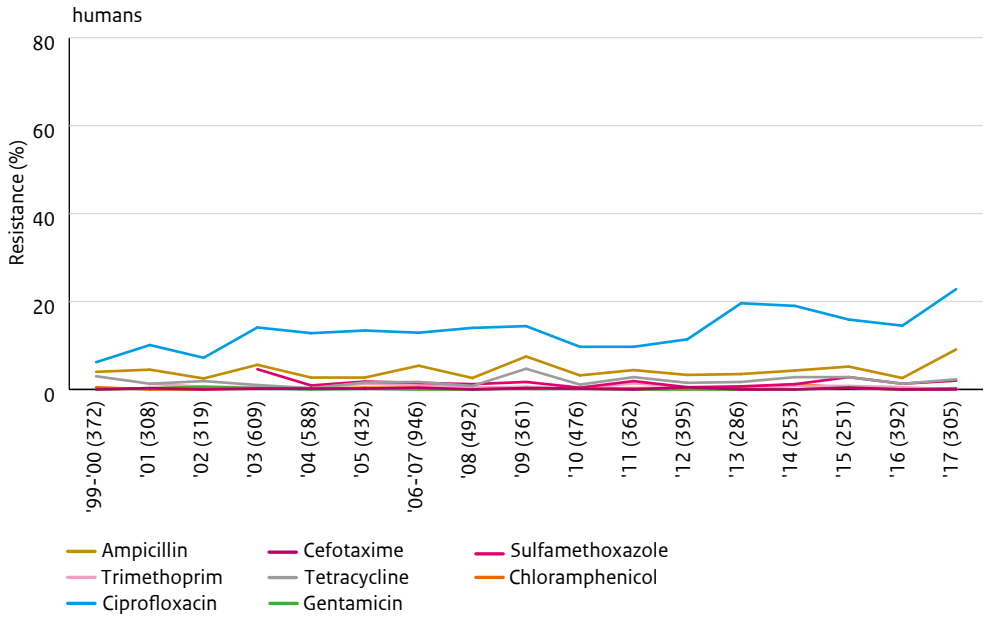
Since 2016, S. Java was not the most predominant serovar isolated in broiler production anymore, as it was in the period before 2015. Figure S03 shows resistance proportions of human and poultry isolates of S. Java. Since 2012, the resistance proportions seem to fluctuate, and a real increasing or decreasing trend cannot be seen. Resistance to trimethoprim was 100%, like in former years. Like in 2016, resistance to chloramphenicol was not detected. The resistance level for ciprofloxacin further increased to 58.8% in 2017.

All 20 tested S. Java strains, isolated from human infections, were trimethoprim susceptible and therefore not considered to be related to the clone spreading in Dutch poultry.

Salmonella from chicken meat, other meat sources and herbs and spices

Table S06 and Figure S04 show resistance data of *Salmonella* isolates from raw meat (chicken and other), herbs and spices (due to oversampling S. Heidelberg was not included in the analysis in Figure S04). S. Heidelberg (54%) was the most abundant serovar found in imported chicken meat in 2017, followed by S. Paratyphi B variation Java (11%) and S. Infantis (8%). Isolates from other meat samples were resistant against a fewer number of antimicrobials and at lower levels than isolates from chicken meat. Resistance proportions for the quinolones (ciprofloxacin and nalidixic acid) were very high in isolates from chicken meat, especially in imported meat (89.2% and 86.3% respectively); resistance proportions in isolates from other meat samples were a bit lower than in 2016, but the number of isolates was low. Borderline resistance to tigecycline was observed in one S. Infantis isolate (6,3%) from retail meat and in eighteen S. Heidelberg (17,6%) and one S. Schwarzengrund isolate (1,0%) all from imported chicken meat samples. Like in S. Typhimurium, MIC-values were slightly elevated. Tigecycline resistance was not detected in samples from other meat. Resistance to cephalosporins (cefotaxime and ceftazidime) was not detected in chicken meat samples from retail and other meat samples, but was very frequently found in isolates from imported chicken meat samples (67.6% for both drugs).
Only 8 strains were isolated from "other products" (herbs, spices, sea food). Resistance proportions in Table S06 are therefore not representative for those products in general. Resistance to quinolones (ciprofloxacin and nalidixic acid), ampicillin and tetracycline were 12.5% (1 of 8 isolates). For the other antimicrobials no resistance was detected in the isolates from other products.

Figure S02 Trends in resistance (%) of S. Enteritidis isolated from humans from 1999 - 2017.



The overall resistance proportions of *Salmonella* from poultry products over the years are shown in Figure S04. Resistances fluctuate since 2001, with an increasing trend for ciprofloxacin; the resistance proportion for tetracycline also increased since 2001, but is decreasing since 2015. In 2013 a substantial reduction in resistance proportions was observed for most antimicrobials. However, after 2013 resistance proportions tended to increase again for sulfamethoxazole, ciprofloxacin, tetracycline, ampicillin and cefotaxime, with a slight decrease for most of them in 2016 and 2017. The increase in 2014/2015 could reflect the relatively high proportion of strains from imported poultry products included. It should be noticed that the fluctuating resistance proportions during the years, could be influenced by the varying proportions of imported products sampled per year.

Figure S03 Trends in resistance (%) of S. Paratyphi variant Java isolated in humans and broilers from 1999 - 2017.

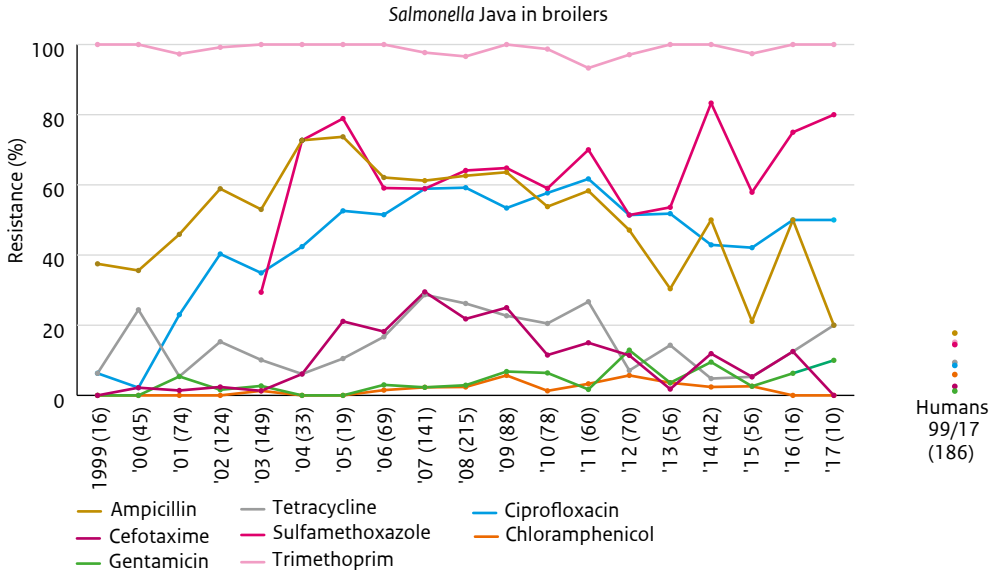


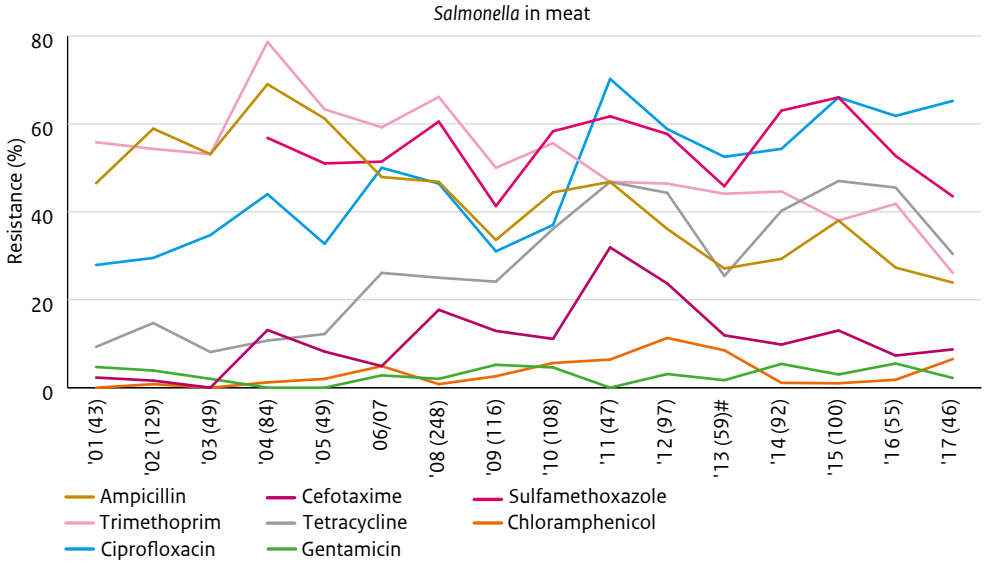
Table S06 Resistance (%) of *Salmonella* enterica isolated from different types of raw meat, herbs, spices and seafood in the Netherlands in 2017.

	Chicken Retail N = 16	Chicken Imported N = 102	Other meat[a] N = 17	Other products[b] N = 8
Ampicillin	25.0	71.6	5.9	12.5
Cefotaxime	0.0	67.6	0.0	0.0
Ceftazidime	0.0	67.6	0.0	0.0
Gentamicin	6.3	5.9	0.0	0.0
Tetracycline	56.3	74.5	0.0	12.5
Sulfamethoxazole	75.0	77.5	0.0	0.0
Trimethoprim	56.3	5.9	5.9	0.0
Ciprofloxacin	68.8	89.2	17.6	12.5
Nalidixic acid	68.8	86.3	17.6	12.5
Chloramphenicol	0.0	3.9	0.0	0.0
Azithromycin	6.3	5.9	0.0	0.0
Meropenem	0.0	0.0	0.0	0.0
Tigecycline	6.3	18.6	0.0	0.0

a. Other meat includes pork (n = 4) , beef (n = 3), lamb (n = 3), turkey (n = 2), frog (n = 4) and cangaroo (n = 1).

b. Other products includes spices and herbs (n = 6) and seafood (n = 2).

Figure S04 Trends in resistance (%) of *Salmonella* enterica isolated from poultry meats in the Netherlands from 2001-2017.



 # Due to an oversampling, S. Heidelberg was excluded from the analysis in 2013 (see Nethmap/MARAN2014).

3.1.2 Campylobacter

This chapter describes the occurrence and trends in antimicrobial resistance in *Campylobacter jejuni* and *C. coli*. Isolates were sampled from food animals, meat and from humans suffering from acute gastroenteritis. Data on human isolates were derived from sixteen regional public health laboratories. As a result of prioritization and changes in legislation, from 2014 onwards the surveillance of antimicrobial resistance in *Campylobacter* focusses mainly on poultry (and poultry meat products). In addition to broilers, in 2017 also *C. jejuni* isolates from faecal samples collected at beef cattle farms, and *C. coli* isolates from pig caecal samples were tested for resistance.

The MIC-distributions and resistance percentages for all *Campylobacter jejuni* and *C. coli* strains isolated at WBVR from caecal samples of broilers and pigs in 2017 are presented in table C01. Resistance percentages of *C. jejuni* and *C. coli* isolated from broilers, cattle, pigs and poultry meat are shown in Table C02. Trends in resistance of *C. jejuni* and *C. coli* from broilers and poultry meat products over the last 12 to 16 years are presented in Figures C01 and C02.
National surveillance data from 2002 onwards for *Campylobacter* spp. isolated from humans are shown in Figure C03, and from 2007 onwards in Table C03.

Highlights

1. Proportions of resistance in *C. jejuni* from caecal samples of broilers and meat thereof were traditionally high for quinolones and tetracycline and did not substantially change in 2017, compared to 2016.
2. In *C. jejuni* from faecal samples of beef cattle and *C. coli* from caecal samples of pigs, proportions of resistance were lower.
3. Resistance to macrolides was rarely detected in isolates from livestock and humans and almost exclusively found in *C. coli* isolates from broilers and pigs.
4. Overall, resistance proportions were higher in *C. coli* than in *C. jejuni* isolates.
5. In *C. jejuni* from cattle, resistance percentages were highest for ciprofloxacin, nalidixic acid and tetracycline, but at much lower levels than in poultry.
6. In. *C. coli* from pigs, high resistance levels were found for streptomycin and tetracycline.
7. Ciprofloxacin resistance in *Campylobacter* isolates from human patients is still high (with an increase in 2017), which is a concern for public health. Resistance to erythromycin, first choice antibiotic in human medicine for campylobacteriosis, remained low.
8. For *C. jejuni* and *C. coli* from human patients, resistance proportions were higher for all three antimicrobials tested in travel related infections compared to domestically acquired campylobacteriosis.

Resistance proportions

EU legislation on monitoring and reporting of antimicrobial resistance in zoonotic and commensal bacteria (2013/652/EU), implemented in November 2013, includes susceptibility testing of mandatory panels of antimicrobials. Since the start of the monitoring programme of *Campylobacter* spp., six out of twelve antimicrobials (ampicillin, chloramphenicol, clarithromycin, tulathromycin, sulfamethoxazole and neomycin) are no longer included. Most of the remaining antimicrobials in the panel: ciprofloxacin, gentamicin, erythromycin and tetracycline, represent antimicrobial classes, which are used in human medicine for treatment of campylobacteriosis.

In 2017, the highest proportions of resistant *C. jejuni* and *C. coli* from broilers and pigs were detected for tetracycline and the quinolones ciprofloxacin and nalidixic acid, and in *C. coli* isolates from pigs also for streptomycin (Table C01). Table C02 shows that resistance percentages were high in isolates from broilers and poultry meat (both *C. jejuni* and *C. coli*), and lower for *C. jejuni* isolates from beef cattle and *C. coli* isolates from pigs.

In recent years, in broilers and poultry meat *C. jejuni* resistant to erythromycin, streptomycin and gentamicin were only incidentally found. In 2017, resistance to these antimicrobials could not be detected in isolates from broilers, as was the case for gentamicin in poultry meat. Resistance to tetracycline was more frequently detected in both poultry meat and broilers in 2017 (59.2% in broilers and 55.8% in poultry meat). Resistance to ciprofloxacin showed fluctuation over the years and was over 60% since 2014 for broilers and poultry meat (Figure C01).

The resistance levels in *C. coli* isolates from broilers and poultry meat showed more fluctuation over years than in C. *jejuni*, which is affected by the lower number of isolates in the survey (Figure C02). Resistance in *C. coli* from broilers and poultry meat could not be detected for gentamicin. However, resistance in *C. coli* was low for erythromycin and streptomycin in 2016, but was more frequently found in 2017 for both broilers and poultry meat. Resistance percentages for ciprofloxacin in broilers have been fluctuating since 2001, but increased to a very high level (94.4%) in 2017. However, because of the low number of *C. coli* isolates tested in 2016 (N = 23) and 2017 (N = 36) these results might not be very representative. Resistance to tetracycline in broilers seem to follow the same trend as ciprofloxacin resistance, at approximately equal percentages (Figure C02); in poultry meat the resistance level for tetracycline increased to 69.4% in 2017.

Overall, resistance proportions were higher in *C. coli* than in *C. jejuni* isolates (Table C01 and C02).

Table C02 shows that resistance against gentamicin was not detected in any of the *C. jejuni* and *C. coli* isolates. Resistance against streptomycin and erythromycin was at low levels for the *C. jejuni* isolates from broilers, poultry meat and beef cattle. A high resistance rate against streptomycin was detected in *C. coli* isolates from pigs (73.5%). Lower levels of resistance against streptomycin were detected in *C. coli* isolates from broilers (8.0%) and poultry meat (11.1%).

Table C01 MIC distribution (in %) for *Campylobacter jejuni* (N = 157) and *C. coli* (N = 50) isolated from caecal samples of broilers and *C. coli* (N= 83) from pigs in 2017.

C. jejuni, *broilers*	MIC (%) distribution mg/L												R%	95% CI
(N = 157)	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256		
Ciprofloxacin	29.9	2.5	0.0	0.0	0.0	1.9	31.8	26.8	7.0				67.5	59.6 - 74.7
Nalidixic acid				0.0	4.5	29.3	0.0	0.0	0.0	0.0	66.2		66.2	58.3 - 73.6
Erythromycin				68.8	29.3	1.9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0 - 2.3
Gentamicin	68.2	31.2	0.6	0.0	0.0	0.0	0.0	0.0					0.0	0 - 2.3
Streptomycin		3.8	54.8	40.8	0.6	0.0	0.0	0.0					0.0	0 - 2.3
Tetracycline			37.6	3.2	0.0	0.0	0.0	5.1	2.5	7.6	43.9		59.2	51.1 - 67.0

C. coli, *broilers*	MIC (%) distribution mg/L												R%	95% CI
(N = 50)	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256		
Ciprofloxacin	16,0	6,0	0,0	0,0	0,0	22,0	36,0	20,0	0,0				78,0	64,0 - 88,5
Nalidixic acid				0,0	0,0	14,0	8,0	0,0	0,0	0,0	78,0		78,0	64,0 - 88,5
Erythromycin				66,0	16,0	2,0	0,0	0,0	0,0	0,0	0,0	16,0	16,0	7,2 - 29,1
Gentamicin	4,0	76,0	20,0	0,0	0,0	0,0	0,0	0,0					0,0	0 - 7,1
Streptomycin		0,0	2,0	78,0	12,0	0,0	0,0	2,0	6,0				8,0	2,2 - 19,2
Tetracycline			16,0	4,0	0,0	0,0	0,0	0,0	0,0	0,0	80,0		80,0	66,3 - 90,0

C. coli, *pigs*	MIC (%) distribution mg/L												R%	95% CI
(N = 83)	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256		
Ciprofloxacin	74,7	9,6	0,0	0,0	0,0	1,2	6,0	4,8	3,6				15,7	8,6 - 25,3
Nalidixic acid				0,0	0,0	47,0	30,1	7,2	0,0	1,2	14,5		15,7	8,6 - 25,3
Erythromycin				51,8	36,1	6,0	0,0	0,0	0,0	0,0	0,0	6,0	6,0	2,0 - 13,5
Gentamicin	6,0	62,7	31,3										0,0	0 - 4,4
Streptomycin		0,0		13,3	12,0	1,2	1,2	24,1	48,2				73,5	62,7 - 82,6
Tetracycline			7,2	2,4	2,4	1,2	2,4	1,2	1,2	7,2	74,7		88,0	79,0 - 94,1

Table C02 Resistance percentages of C. *jejuni* and C. *coli* isolated from faecal samples of broilers, cattle and pigs and from poultry meat in 2017.

	C. jejuni			*C. coli*		
	Broilers	Poultry meat	Cattle	Broilers	Poultry meat	Pigs
N	157	77	90	50	36	83
Ciprofloxacin	67,5	64,9	23,3	78,0	94,4	15,7
Nalidixic acid	66,2	62,3	25,6	78,0	94,4	15,7
Erythromycin	0,0	0,0	1,1	16,0	16,7	6,0
Gentamicin	0,0	0,0	0,0	0,0	0,0	0,0
Streptomycin	0,0	2,6	1,1	8,0	11,1	73,5
Tetracycline	59,2	55,8	17,8	80,0	69,4	88,0

Quinolones

The high proportion of *Campylobacter* spp. isolates from animal origin resistant to the quinolones (Figures C01 and C02) and especially from human patients (Figure C03) is a public health concern.
The proportion of *C. jejuni* isolates from broilers resistant to quinolones remains at a continuous high level in the last decade with 67.5% in 2017. The proportion of quinolone resistance in *C. jejuni* from poultry meat is comparably high with 64.9% in 2017. Ciprofloxacin resistance proportions in *C. jejuni* isolates from beef cattle and from *C. coli* isolates from pigs were at medium levels in 2017 (23.3% and 15.7% respectively). For *C. coli* from pigs resistance was higher than in 2013 (6.1%).
A continuation of high levels of ciprofloxacin resistance were also observed in the *C. coli* isolates from broilers with 78% in 2017. The proportion of resistance of *C. coli* isolates from poultry meat strongly fluctuates in time due to the low number of isolates included in the survey. Nevertheless, the proportion of resistance to quinolones was remarkably high in 2017: 94.4% for both ciprofloxacin and nalidixic acid. The resistance levels for fluoroquinolone in human campylobacter isolates were also high (61.6%), which was approximately at the same level as in the years 2014-2016 (58.3 - 61.4%).

Macrolides

Erythromycin, or other macrolides (clarithromycin), are the first-choice drugs for the treatment of campylobacteriosis in humans. The proportion of resistance to macrolides reported in animals and humans was low: it could not be detected in *C. jejuni* from caecal samples of broilers in 2017, and 2.0% of human isolates from 2013-2017 were classified as resistant. It should be noted that for human isolates a lower breakpoint for resistance has been applied for erythromycin (≥ 1.5-2.0 mg/L); for animal and meat isolates the EUCAST epidemiological cut-off values were used (> 4 mg/L for C. *jejuni*, and > 8 mg/L for C. *coli*).
Like the years before, also in 2017, erythromycin resistance was very rare in C. *jejuni* isolates, with no resistance in broilers and poultry meat, and in one erythromycin resistant C. *jejuni* isolate (1.1%) isolated in a faecal sample from a beef cattle farm (Table C02). Erythromycin resistance was detected in *C. coli* from caecal samples of broilers (16.0%) and in poultry meat (16.7%) and in slaughter pigs (6%).

Figure C01 Trends in resistance (%) of *Campylobacter jejuni* isolated from broilers and poultry meat in the Netherlands.

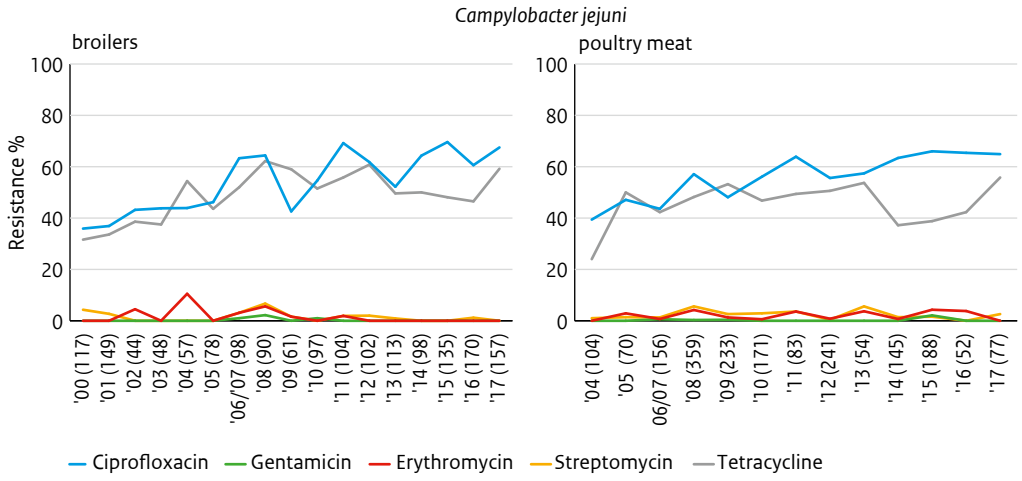


Figure C02 Trends in resistance (%) of *Campylobacter* coli isolated from broilers and poultry meat in the Netherlands.

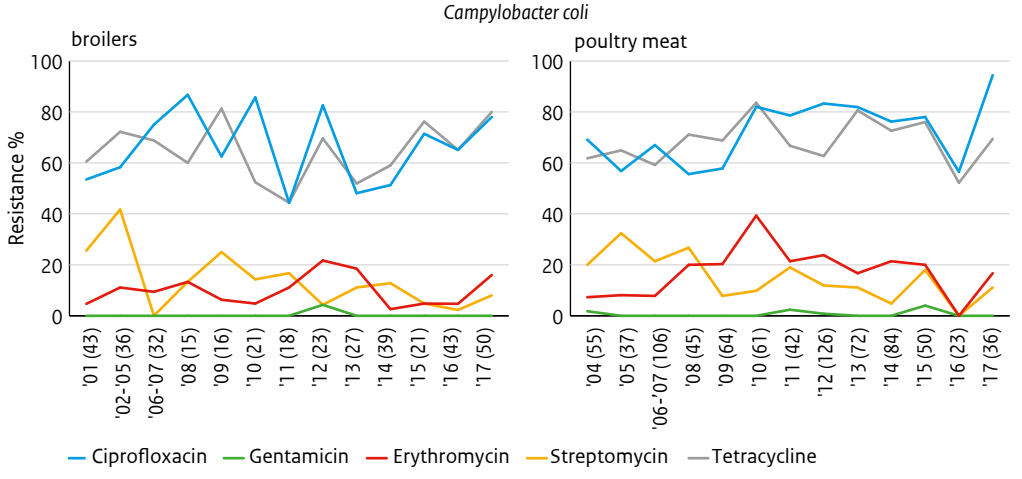


Table C03 Domestically acquired and travel related resistance in C. *jejuni* and *C. coli* isolated from humans from 2007 - 2017 from all 16 Public Health Services (PHLS) covering >50% of the Dutch population.

	2007-2012							
	Domestically acquired				Travel related			
	C. jejuni		*C. coli*		*C. jejuni*		*C. coli*	
	N	R%	N	R%	N	R%	N	R%
Fluoroquinolone	18374	51.3	1340	51.5	958	65.4	101	60.4
Tetracycline	10875	21.9	897	31.3	290	31.7	49	28.6
Erythromycin	15261	2.2	1131	7.5	726	3.9	80	12.5

	2013-2017							
	Domestically acquired				Travel related			
	C. jejuni		*C. coli*		*C. jejuni*		*C. coli*	
	N	R%	N	R%	N	R%	N	R%
Fluoroquinolone	13673	58.7	970	63.1	952	76.7	111	75.7
Tetracycline	9259	41.0	625	62.2	584	58.4	64	65.6
Erythromycin	11956	2.0	802	13.7	858	3.7	102	32.4

	Campylobacter spp. (R%)					
	2017	2016	2015	2014	2013	2007/12
Fluoroquinolone	61.6	58.3	61.4	60.6	57.6	51.9
Tetracycline	47.6	42.0	42.3	43.9	38.5	30.0
Erythromycin	3.5	2.6	2.9	3.2	3.2	2.7

Broiler chickens and poultry meat

In *Campylobacter* from poultry, resistance profiles were determined for isolates recovered from broilers as well as from chicken meat samples. In 2017 no samples were collected from laying hens, ducks and turkey meat.

Table C02 shows that the proportions of resistance for tetracycline and the quinolones in *C. jejuni* isolates from poultry meat were at the same high level as for the isolates from caecal samples of broilers. The proportion of resistance for the *C. coli* isolates from broilers were also high for tetracycline and quinolones. Resistance to gentamicin was not observed in *C. jejuni* and *C. coli* isolates. Resistance to erythromycin and streptomycin was rarely observed in *C. jejuni* and more frequently found in *C. coli*. Moreover, streptomycin resistance was commonly detected in *C. coli* isolates from pigs (73.5%).

In general, higher resistance rates were observed for most antimicrobials in *C. coli* isolates from broilers and poultry meat, compared to *C. jejuni* isolates from the same sources. Overall, Figure C01 and Figure C02 show similar trends in resistance proportions of both *C. jejuni* and *C. coli* in broilers and poultry meat.

Cattle and pigs

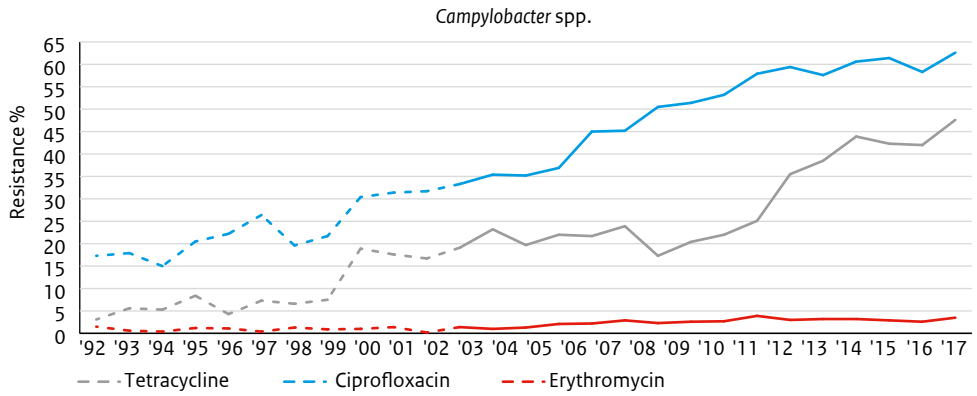
In 2017 *C. jejuni* isolates from beef cattle and *C. coli* isolates from pigs were tested for antimicrobial resistance. Like in the other animal species, resistance proportions were highest for ciprofloxacin, nalidixic acid and tetracycline, but at much lower levels in the cattle samples (23.3% for ciprofloxacin, 25.6% for nalidixic acid and 17.8% for tetracycline). Resistance against ciprofloxacin and nalidixic acid were also at lower levels in pig samples (for both 15.7%), but at a high level for tetracycline (88.0%) (Table C02). A high resistance rate (73.5%) was also detected in *C. coli* isolates from pigs.

Campylobacter in humans

Data on resistance levels are available for ciprofloxacin, tetracycline and erythromycin are summarized in Table C03 and Figure C03. Figure C03 shows a continuously increasing trend of ciprofloxacin and tetracycline resistance in *Campylobacter* spp. isolated from human patients, with a slight decrease for tetracycline in 2015 and 2016, and for ciprofloxacin in 2016, but again an increase in 2017. Resistance to erythromycin stabilized around 3% since 2011.

Table C03 shows resistance levels for *Campylobacter* spp. isolates, specified according to the most probable infection route, i.e. whether the infection was acquired domestically or abroad. Resistance levels were higher for all three antimicrobials in travel related infections compared to those domestically acquired for *C. jejuni* isolates. For *C. coli* this was also the fact, but with a smaller difference between travel related and domestically acquired infections. However, these percentages were based on a relatively low number of isolates.

Figure C03 Trends in resistance (%) of *Campylobacter* spp. isolated from humans between 1992 and 2002 at the regional Public Health. Laboratories (PHLS) of Arnhem and Heerlen covering 990.000 inhabitants (400-700 isolates per year). The continuous line represents national surveillance data from 2002 onwards; the average number of strains tested per year was approximately 2400, ranging from 1900-2900.



3.1.3 Shiga-toxin producing *E. coli* (STEC)

Highlights

1. Proportions of resistance to ampicillin, sulfamethoxazole and trimethoprim in human STEC O157 isolates were somewhat higher in 2017, compared to 2016 (10.7% to 16.1% for ampicilline, from 14.7% to 16.1% for sulfamethoxazole, and from 8.0% to 14.5% for trimethoprim). There is an increasing tendency for resistance against these antimicrobials since 2009.
2. Resistance to the quinolones (ciprofloxacin and nalidixic acid) was detected in 3.2% of human STEC O157 isolates.
3. For the first time since seven years one cefotaxime resistant, ESBL-producing isolate was detected.

Shiga-toxin producing *E. coli* O157 (STEC O157) isolates from humans (N = 62) were tested for susceptibility. Table STEC01 shows the MIC results for all *E. coli* O157 isolates from humans; Figure STEC01 presents the trends over time.

Human STEC O157 isolates

Resistance proportions of human isolates showed a tendency to increase for ampicillin, tetracycline and trimethoprim since approximately 2009, whereas resistance against sulfamethoxazole was high since 2009, but fluctuating (Figure STEC01). After a decrease in 2016 for ampicillin, sulfamethoxazole and trimethoprim, levels of resistance increased in 2017 (from 10.7% to 16.1% for ampicilline, from 14.7% to 16.1% for sulfamethoxazole, and from 8.0% to 14.5% for trimethoprim). Resistance for ciprofloxacin and nalidixic acid was not detected in 2015 and 2016, but was 3.2% for both antimicrobials in 2017. For the first time since seven years, a cefotaxime resistant, ESBL-producing isolate was detected harbouring a $bla_{\text{CTX-M-15}}$ gene.

Figure STEC01 Trends in resistance (in %) of *E. coli* STEC O157 isolated from humans in the Netherlands from 1999-2017.

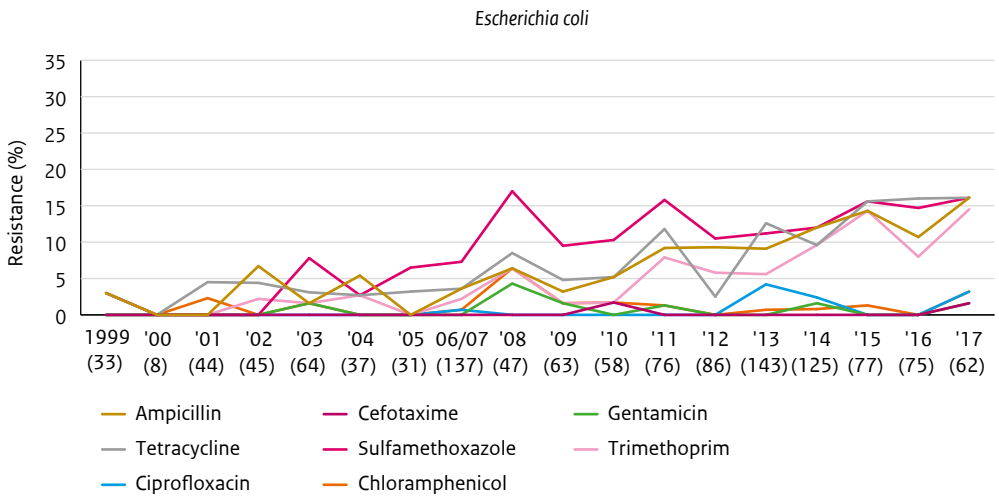


Table STEC01 MIC distribution (in %) and resistance percentages (R%) for *E. coli* STEC O157 (N=62) isolated from humans the Netherlands in 2017.

E. coli	MIC (%) distribution mg/L																	R%	95% CI
N = 62	0.015	0.03	0.06	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	512	1024	2048	
Ampicillin								1.6	77.4					16.1					16.1	8.0 - 27.7
Cefotaxime					98.4					1.6									1.6	0 - 8.7
Ceftazidime						98.4				1.6									1.6	0 - 8.7
Gentamicin						75.8	22.6					1.6							1.6	0 - 8.7
Tetracycline								32.3	51.6				3.2	12.9					16.1	8.0 - 27.7
Sulfamethoxazole										83.9								16.1	16.1	8.0 - 27.7
Trimethoprim					85.5								14.5						14.5	6.9 - 25.8
Ciprofloxacin	45.2	51.6					1.6		1.6										3.2	0.4 - 11.2
Nalidixic acid									91.9	4.8					3.2				3.2	0.4 - 11.2
Chloramphenicol										83.9	12.9				3.2				3.2	0.4 - 11.2
Azithromycin*									83.9	16.1									0.0	0 - 5.8
Colistin							100												0.0	0 - 5.8
Meropenem		98.4	1.6																0.0	0 - 5.8
Tigecycline					88.7	11.3													0.0	0 - 5.8

The white areas indicate the dilution range tested for each antimicrobial agent. Values above this range indicate MIC values > the highest concentration in the range. Values at the lowest concentration tested indicate MIC-values ≤ the lowest concentration in the range. Vertical bars indicate the epidemiological cut-off values, used as breakpoints. Dashed bars indicate the clinical breakpoints.

3.2 Commensal indicator organisms

This chapter describes the susceptibility profiles of commensal bacteria from the gastro-intestinal tract of food-producing animals and meat products. The selection pressure as a result of the use of antibiotics in animals, is reflected in the level of antimicrobial resistance in bacteria inhabiting the intestinal tract, especially over time. For this purpose, *E. coli* is included as indicator organisms for the Gram-negative flora. As a result of less priority for including enterococci in the surveillance, no enterococci were tested in 2017.

Isolation of bacteria from the intestine of randomly picked food-producing animals at slaughter aims to detect the occurrence and trends in resistance at the bacterial population level in food animals as prescribed by EFSA [1].
This monitoring is conducted in slaughter pigs and broilers since 1998. Resistance in isolates from both dairy cattle, veal calves and meat samples have been included from 2005 onwards. In the years 2010 and 2011 samples of individual dairy cattle were collected at slaughter houses, in all other years pooled or individual faecal samples were collected at dairy farms. Monitoring programs in veal calves at farms stopped in 2012. From then, samples from individual veal calves were collected at slaughterhouses and resistance levels were reported separately for white and rosé veal calves.

It should be noted that the sampling strategies used are inherently insensitive to detect resistance at the population level, as only one randomly selected isolate from a single sample collected from one animal per epidemiological unit (herd or flock) is tested for susceptibility. The total number of isolates is intended to represent the *E. coli* population of each animal species of the entire country. One per cent resistance in e.g. *E. coli* indicates that in all animals of that animal species 1% of the *E. coli* bacteria are resistant. This means that the absence of resistance in these datasets does not exclude the possibility that resistance is present in relatively small numbers in individual animals.

[1] Report from the Task Force on Zoonoses Data Collection including guidance for harmonized monitoring and reporting of antimicrobial resistance in commensal Escherichia coli and Enterococcus spp. from food animals. http://www.efsa.europa.eu/en/efsajournal/pub/141r.htm.

3.2.1 *Escherichia coli*

This chapter presents information on resistance in *E. coli*, as indicator organism for the occurrence and trends in resistance in Gram-negative bacteria in the gastro-intestinal tract of food-producing animals in the Netherlands.
In 2014, the EU legislation on monitoring and reporting of antimicrobial resistance in zoonotic and commensal bacteria (2013/652/EU) was implemented. This includes susceptibility testing with mandatory panels of antimicrobials. Results are interpreted with epidemiological cut-off values (ECOFF's) according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST).

Highlights 2017

1. In 2017, resistance proportions of indicator *E. coli* in caecal samples showed a tendency to decrease in broilers, to stabilize in pigs, and showed a slight increase in veal calves. In dairy cattle the resistance proportions remained at a constant low level.
2. As in former years, resistance proportions in *E. coli* from chicken and turkey meat, were substantially higher than in pork and beef
3. The proportion of *E. coli* isolates resistant to third-generation cephalosporins was low in faecal samples from broilers and pigs and not detected in dairy cattle and veal calves.
4. Although resistance to fluoroquinolones is decreasing, it was still commonly present in indicator *E. coli* from caecal samples of broilers and meat thereof.
5. Among indicator *E. coli* from animals and meat, resistance levels to ampicillin, tetracycline, sulfamethoxazole and trimethoprim were still high in broilers, pigs, veal calves and chicken and turkey meat.
6. Levels of resistance in *E. coli* from caecal samples of rosé veal calves were substantially lower than those from white veal calves for almost all antibiotics tested.

Resistance levels

Table Eco01 shows resistance levels of a total of 1194 *E. coli* isolates obtained in caecal samples from broilers, pigs, veal calves and faecal samples of dairy cows , presented as MIC-distributions. Table Eco02 presents resistance percentages per animal species. Trends in resistance levels from 1999 to 2017 are shown in Figure Eco01 and information on trends in multidrug resistance is shown in Figure Eco02. Resistance percentages of 452 *E. coli* isolates collected from raw chicken and turkey meat, pork, beef and veal products are presented in Table Eco03. Figure Eco03 shows trends in resistance of *E. coli* in the Netherlands from 2002 to 2017 isolated from raw meat products of poultry, turkey, pork and beef.

For most drugs or drug classes there were notable variations in resistance levels between the different animal species (Table Eco02). Like the years before, highest levels were found in broilers, slaughter pigs and white veal calves, lower levels in rosé veal calves, and hardly any resistance was observed in isolates from dairy cattle.
Overall, the highest resistance levels were seen for ampicillin, tetracycline, sulfamethoxazole and trimethoprim. These drug classes are the most frequently used in veterinary medicine in The Netherlands.

Table Eco01 MIC distribution (in %) and resistance percentages (R%) for all *E. coli* (N=1194) isolated as indicator organism from intestines of food producing animals in the Netherlands in 2017.

E. coli	MIC (%) distribution mg/L																	R%	95% CI
N = 1194	0.015	0.03	0.06	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	512	1024	2048	
Ampicillin							1.9	28.5	44.3	4.4	0.2			20.8					20.9	18.7- 23.4
Cefotaxime					99.5	0.1				0.4									0.5	0.2 - 1.1
Ceftazidime						99.5	0.1	0.2		0.1	0.2								0.5	0.2 - 1.1
Gentamicin						60.3	31.1	6.2	0.3	0.3	0.8	0.5	0.7						2.4	1.6 - 3.5
Tetracycline								58.4	11.7	0.3	0.3	0.9	9.5	18.8					29.6	27.0 - 32.2
Sulfamethoxazole										74.5	0.2			0.1	0.1	0.3		24.9	25.4	22.9 - 28.0
Trimethoprim					29.8	46.8	3.1	0.2					20.2						20.1	17.9 - 22.5
Ciprofloxacin	73.5	16.4	0.0	0.6	5.5	2.3	0.6	0.3	0.2	0.3	0.3								10.1	8.4 - 11.9
Nalidixic acid									89.4	1.1	0.5	0.2	0.7	4.5	3.6				9.0	7.4 - 10.7
Chloramphenicol										85.8	5.2	1.2	0.6	3.3	3.9				9.0	7.4 - 10.7
Azithromycin*								2.3	37.1	56.4	3.9	0.1	0.2	0.1					0.3	0.1 - 0.9
Colistin							99.9	0.1											0.0	0 - 0.3
Meropenem		99.8	0.2																0.0	0 - 0.3
Tigecycline					78.9	17.4	3.7												0.0	0 - 0.3

The white areas indicate the dilution range tested for each antimicrobial agent. Values above this range indicate MIC values > the highest concentration in the range. Values at the lowest concentration tested indicate MIC-values ≤ the lowest concentration in the range. Vertical bars indicate the epidemiological cut-off values (ECOFF), used as breakpoints. If available, dashed bars indicate the clinical breakpoints. For ampicillin, chloramphenicol and colistin the ECOFF and clinical breakpoint are identical.

** tentative ECOFF set by EURL established by EFSA data*

Table Eco02 Resistance (in %) of *E. coli* isolated from faecal samples of broilers, pigs, dairy cows, white veal calves and rosé veal calves in the Netherlands in 2017.

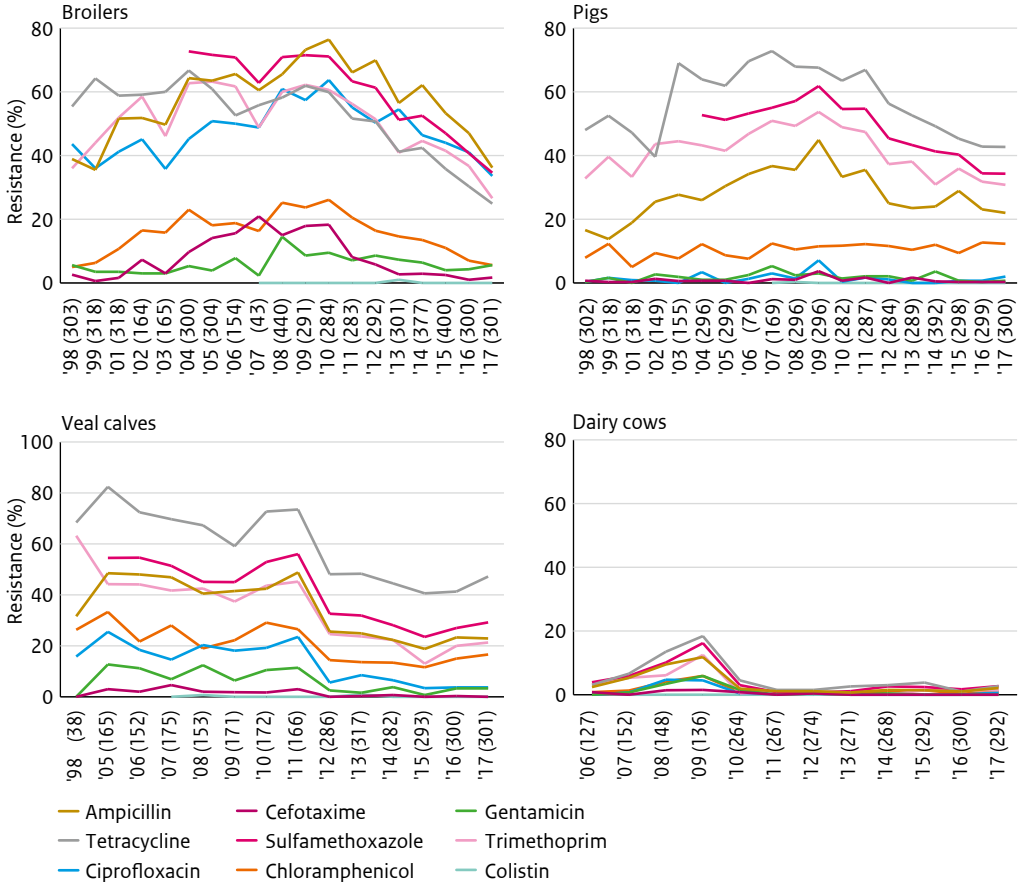
Faecal samples	Broilers	Pigs	Dairy	Veal calves	
	N = 301	N = 300	N = 292	White, N = 209	Rosé, N = 92
Ampicillin	36.2	22,0	2,1	27,8	12,0
Cefotaxime	1.7	0,3	0,0	0,0	0,0
Ceftazidime	1.7	0,3	0,0	0,0	0,0
Gentamicin	5.6	0,7	0,0	3,8	2,2
Tetracycline	24.9	42,7	2,7	61,2	15,2
Sulfamethoxazole	34.6	34,3	2,7	37,8	9,8
Trimethoprim	26.6	30,8	1,4	28,2	5,4
Ciprofloxacin	33.6	2,0	0,7	5,3	0,0
Nalidixic acid	30.6	1,3	0,7	4,3	0,0
Chloramphenicol	5.6	12,3	1,0	21,5	5,4
Azithromycin	0.7	0,7	0,0	0,0	0,0
Colistin	0,0	0,0	0,0	0,0	0,0
Meropenem	0,0	0,0	0,0	0,0	0,0
Tigecycline	0,0	0,0	0,0	0,0	0,0

Quinolones

The proportion of isolates resistant to quinolones were highest in *E. coli* from broilers: 33.6% resistance to ciprofloxacin and 30.6% resistance to nalidixic acid. This was a decrease compared to previous years (going down from 54% resistance for both drugs in 2013), although the resistance levels are still high. High level resistance (MIC >1 mg/L) to ciprofloxacin in broilers was detected in 3.6% (11/301) of the isolates in 2017. In 2017, resistance to ciprofloxacin was 5.3% in *E. coli* isolates from white veal calves, 2.0% in pigs, very low in isolates from dairy cows and not detected in isolates from rosé veal calves.

Resistance to quinolones in *E. coli* from meat was tested for chicken and turkey meat samples and pork, beef and veal, sampled from retail in The Netherlands. In 2017, no samples from imported meat were analysed. Resistance in chicken products at retail was a bit higher than in 2016: the percentage of *E. coli* with resistance to ciprofloxacin and nalidixic acid was 26.9% (22.4% in 2016) and 23.1% (20.9 % in 2016), respectively. In isolates from turkey products the resistance percentages were a little lower than in 2017 (30.6% and 25.0% for ciprofloxacin and nalidixic acid respectively). Figure Eco03 shows that resistance levels in pork and beef was low, as in former years. The resistance percentages of *E. coli* from meat were somewhat higher for ciprofloxacin than for nalidixic acid. This is most probably due to the increase of plasmid mediated quinolone resistance (PMQR) exhibiting resistance to ciprofloxacin, but not to nalidixic acid.

Figure Eco01 Trends in proportion of resistance (%) of *E. coli* isolated from broilers, slaughter pigs, veal calves and dairy cattle in the Netherlands from 1998 - 2017.

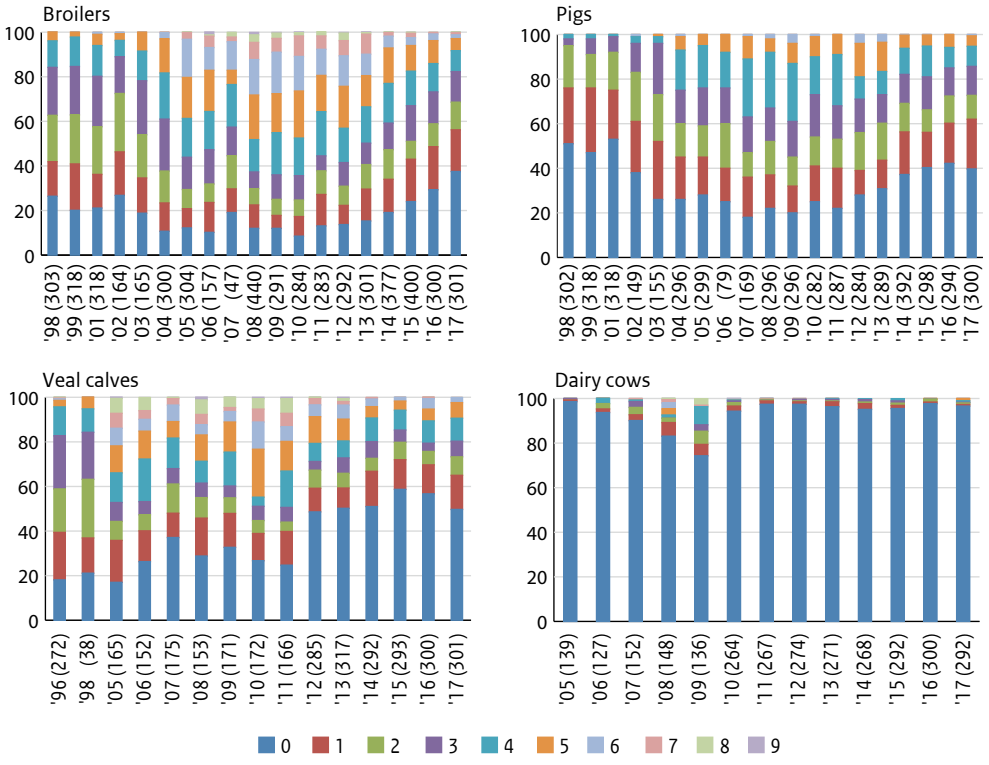


Cefotaxime resistance

The proportion of isolates resistant to third generation cephalosporins (cefotaxime and ceftazidime), indicative of ESBL/pAmpC producing *E. coli*, was very low in broilers and pigs. Cefotaxime resistance was not detected in isolates from dairy cows and veal calves (white and rosé). Resistance proportions for *E. coli* were 1.7% in broilers and 0.3% in pigs for both cefotaxime and ceftazidime. The 1.7% cefotaxime resistance proportion in broiler isolates was at a similar level as in 2016 (1.0%), which indicates a stabilised low level after a decreasing trend from 2013 to 2016 (Figure Eco01).

For the first time since 2014, resistance to cefotaxime in randomly isolated commensal *E. coli* obtained from chicken meat samples from retail could not be detected (Table Eco03). The proportion of isolates with cefotaxime resistance from turkey meat samples from retail was a bit higher than in 2016 (from

Figure Eco02 Resistance (%) to 0 - 9 antimicrobial classes among *E. coli* isolated from broilers, slaughter pigs, veal calves and dairy cattle in the Netherlands from 1998 - 2017.



1.5% in 2016 to 5.6% in 2017). As in 2017 no samples from imported meat (outside EU) were collected, the resistance percentages shown in Figure Eco03 for 2017 cannot be compared with the percentages in former years, which were the total result from imported and retail meat samples.

The absence of finding cefotaxime resistant *E. coli* on chicken meat samples, in randomly isolated strains cultured on non-selective media, suggests that the concentration of *E. coli* resistant to Extended Spectrum Cephalosporins (ESC) on meat decreased. However, the proportion of fresh chicken meat samples in which ESC-resistant *E. coli* were found using selective media slightly increased in 2017 to 31.6% after a decreasing trend from 2014 (67%) – 2016 (26.4%) (see chapter 4). One has to consider the fact that part of the retail meat included in the sampling originates from EU countries outside the Netherlands where resistance prevalences might be higher.

Importantly, the prevalence of broilers produced in the Netherlands carrying ESC-resistant *E. coli* decreased from 50.3% of the animals sampled in 2016 to 32.6% in 2017 (see chapter 4). The decrease in prevalence and concentrations of ESC-resistant *E. coli* on poultry meat is an important finding because it suggests that the exposure of humans to ESC-resistant *E. coli* through contaminated meat is also decreasing. In contrast, in veal calves a second year with relatively high prevalence of animals positive for ESC-resistant *E. coli* was found. In white veal calves the prevalence was highest with 40.5% of the animals sampled (compared to 28.3% in rosé). The prevalence in 2017 of animals positive for ESC-resistance in pigs and dairy cattle were 11.0 and 10.3% respectively.

Broiler chickens

Commensal *E. coli* isolated from caecal samples from broiler chickens showed decreasing resistance to almost all antimicrobials (Table Eco02). Resistance proportions for ampicillin (36.2%), tetracycline (24.9%), sulfamethoxazole (34.6%), trimethoprim (26.6%) and the quinolones ciprofloxacin (33.6%) and nalidixic acid (30.6%) were high, but lower than in 2016. Cefotaxime resistance decreased from 2.5% in 2015 to 1.0% in 2016, but remained stable in 2017 (1.7%) .

Slaughter pigs

Resistance proportions for tetracycline, sulfamethoxazole, trimethoprim and ampicillin in *E. coli* isolates from pigs, sampled in 2017, were 42.7%, 34.3%, 30.8% and 22.0%, respectively, which was approximately at the same level as in 2016. The proportion of isolates resistant to these four antibiotics showed a decreasing tendency since 2011, which stabilized from 2015 onwards. (Figure Eco01). Resistance to the 3rd generation cephalosporins did not change compared to 2015 and 2016 (0.3%).

Veal calves

Resistance data on white and rosé veal calves are reported separately, because of the difference in production systems. White veal calves are fattened on a milk diet with a required minimal uptake of roughage, while rosé veal calves are also fed corn silage, straw or pelleted feed. Most antibiotics are administered during the starting period in both production systems. On average, in white veal calves more antibiotics are used than in rosé calves and rosé calves are slaughtered at an older age, which results in a longer time period with relatively low antibiotic exposure. This results in a difference in resistance levels between the two husbandry types. As in the years before, substantially higher resistance levels were measured for isolates from white , compared to those from rosé veal calves (Table Eco02).

Figure Eco01 illustrates the trends in resistance in *E. coli* isolated from both types of veal calves combined. Resistance levels have been relatively stable over time, with a clear decrease in 2012, which was the year in which the sampling strategy changed from sampling at farm to sampling at slaughterhouse. This has influenced the results from 2012 and onwards, because most antibiotics are used in the young calves and less in the period before slaughter.

The highest resistance levels in 2017 were against tetracycline (61.2% and 15.2% for white and rosé respectively), sulfamethoxazole (37.8% and 9.8%), trimethoprim (28.2% and 5.4%) and chloramphenicol (21.5% and 5.4%).

The ratio of sampled white veal calves versus rosé veal calves changed from 50/50% to 60/40% in 2016, and to 70/30% in 2017, which better reflected the proportions of slaughtered white and rosé calves in The Netherlands in 2016/2017. This explains part, but not all of the apparent increase in resistant rates

of *E. coli* in veal calves in 2016 and 2017 compared to 2015. *E. coli* isolates resistant to 3rd generation cephalosporins were not detected in veal calves in 2017 (TableEc002).

Dairy cattle

Resistance in *E. coli* isolated from dairy cattle is very low compared to resistance proportions observed in pigs, broilers and veal calves (Table Ec002), reflecting the low use of antibiotics in this husbandry system. Resistance proportions were comparable to 2015 and 2016. The overall rates remained below 3%. No resistance to 3rd generation cephalosporins was detected.

Multidrug resistance

Due to the implementation of new antimicrobial susceptibility testing panels for *E. coli*, the data to determine multidrug resistance have been adjusted backwards starting from 2014. For this reason, trends in multidrug resistance should be interpreted with care. Figure Ec002 shows the data with the determined level of multidrug resistance over the years.

In 2017, a decreasing trend in the proportion of multidrug resistant isolates (resistant to three or more classes of antibiotics) was observed in broilers (from 41.0% in 2016 to 31.4% in 2017), but the proportion of multidrug resistance stabilized at relatively high levels in pigs (27.3%) and veal calves (26.7%). In dairy cattle multidrug resistance in *E. coli* was still rarely detected with 2.1% of the isolates showing resistance to three or more classes of antimicrobials. Multidrug resistance in isolates from pigs was at the same level as in 2016 (27.3%).

The increasing tendency of the percentage of completely susceptible *E. coli* isolates from broilers and pigs (Figure Ec002), which was reported for 2016, is ongoing in broilers, but not in isolates from pigs.

E. coli in raw-meat

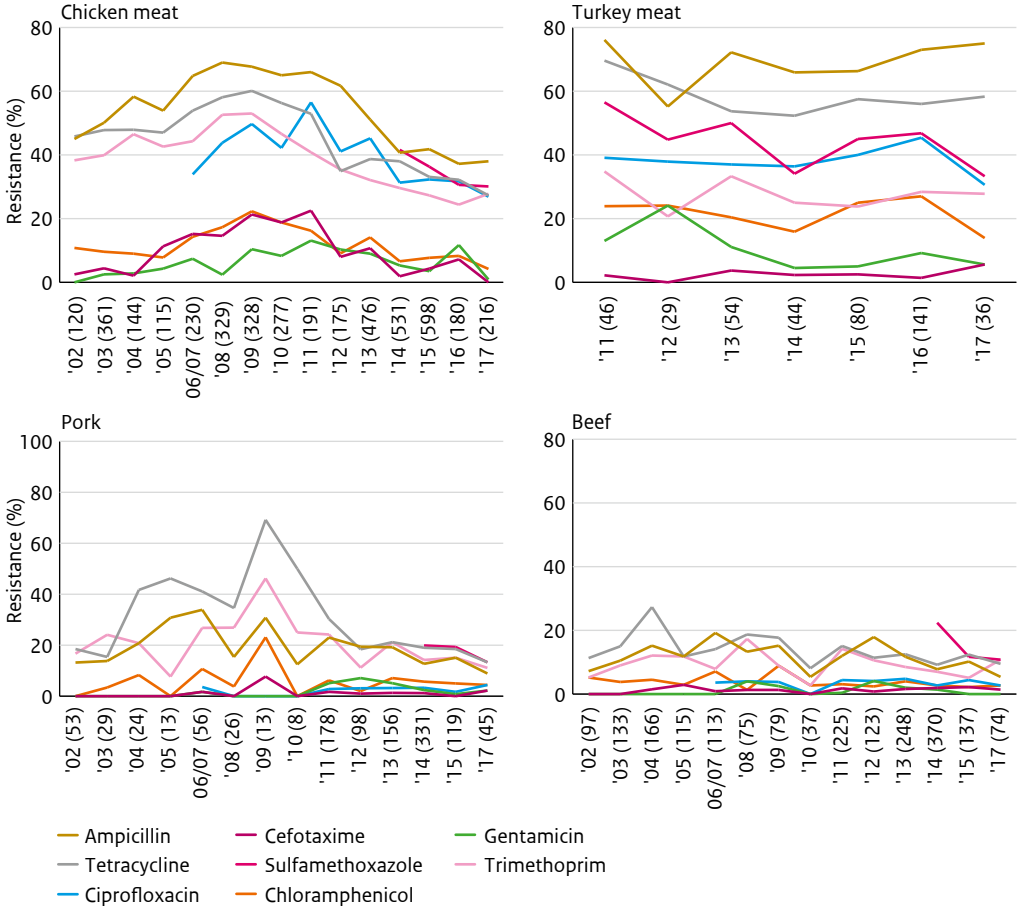
Table Ec003 presents resistance percentages of *E. coli* isolated from raw meat from chicken and turkey, pork, beef and veal, sampled at retail by the Dutch Food and Consumer Product Safety Authority (NVWA). Meat from retail can include meat produced in The Netherlands, but also other EU countries. Meat products imported from outside the EU were not analysed for commensal *E. coli* 2017.

The trends in resistance are presented in Fig Ec003. Resistance rates in chicken meat show a tendency to decrease from 2010 onward, with some slight increases in 2015/2016, but the decreasing trend seems to go on in 2017. In turkey meat, resistance rates have been at a constant high level since 2011, with a decrease in 2017 for sulfamethoxazole, ciprofloxacin and chloramphenicol. In 2017, cefotaxime resistance could not be detected in *E. coli* isolates from chicken meat, but slightly increased in isolates from turkey meat samples, compared to former years. Fluctuations in the resistance rates might be caused by a year-to-year variation in the proportion of retail poultry meat produced within the European Union, but outside of the Netherlands included in the survey.

Table Eco03 Resistance (in %) of *E. coli* isolated from raw chicken meat, turkey meat, pork, beef and veal at retail in the Netherlands in 2017.

Meat products	Chicken	Turkey	Pork	Beef	Veal
	N = 216	N = 36	N = 45	N = 74	N = 81
Ampicillin	38.0	75.0	8.9	5.4	27.2
Cefotaxime	0.0	5.6	2.2	1.4	0.0
Ceftazidime	0.5	2.8	0.0	0.0	1.2
Gentamicin	0.9	5.6	2.2	0.0	1.2
Tetracycline	27.3	58.3	13.3	9.5	38.3
Sulfamethoxazole	30.1	33.3	13.3	10.8	24.7
Trimethoprim	27.8	27.8	11.1	10.8	22.2
Ciprofloxacin	26.9	30.6	4.4	2.7	4.9
Nalidixic acid	23.1	25.0	4.4	2.7	1.2
Chloramphenicol	4.2	13.9	4.4	2.7	7.4
Azithromycin	0.5	2.8	0.0	0.0	2.5
Colistin	1.4	2.8	0.0	0.0	0.0
Meropenem	0.0	0.0	0.0	0.0	0.0
Tigecycline	0.0	0.0	0.0	0.0	0.0

Figure Eco03 Trends in resistance (%) of *E. coli* isolated from raw chicken meat, turkey meat, pork and beef in the Netherlands from 2002 - 2017.



4
Screening for ESBL, AmpC, carbapenemase-producing and colistin-resistant Enterobacteriaceae in food-producing animals and meat in the Netherlands in 2017

Highlights

1. Within the randomly isolated indicator *E. coli* in faecal samples from broilers a continuous low proportion of ESBL/AmpC-producing *E. coli* was observed in the last five years (<3%) and this was confirmed in 2017 (1.7%). No ESBL/AmpC-producing *E. coli* were detected in faecal samples from pigs, veal calves and dairy cattle.
2. Selective culturing in livestock faeces showed a further decrease in the prevalence (% of animal carriers) of ESBL/AmpC-producing *E. coli* in broilers.
3. For the second year in a row, an increase was observed in white and rosé veal calves carrying ESBL/AmpC-producing *E. coli*. 2017 was the first year a higher prevalence was recorded in veal calves than in broilers (36.7% vs 32.6%).
4. The most prevalent ESBL/AmpC gene was $bla_{\text{CTX-M-1}}$ in all animal species. $bla_{\text{CTX-M-15}}$ was found frequently in veal calves and dairy cows (30%). $bla_{\text{CMY-2}}$ in broilers (25%), $bla_{\text{SHV-12}}$, $bla_{\text{TEM-52c}}$ and $bla_{\text{CTX-M-14}}$ followed in prevalence. A comparable gene distribution was observed in corresponding meat samples.
5. Prevalence of ESBL/AmpC-producing *E. coli* in meat in 2017 was 9.6%. After three years of decreasing prevalence (67% to 24% in 2014-2016), in 2017 31.6% of fresh chicken meat samples were found positive, resulting in a similar prevalence as in broilers (32.6%). Imported chicken meat was more frequently positive (56.1%). Also lamb and veal meat were found more frequently positive than in previous years.
8. The proportion of human ESBL/AmpC-producing *Salmonella* in 2017 was 1.8%, confirming a continuous low level (≤2%) since 2014. Most represented ESBL/AmpC genes were $bla_{\text{CTX-M-14b}}$, generally associated with S. Kentucky, $bla_{\text{CTX-M-9}}$ in S. Typhimurium, and $bla_{\text{CMY-2}}$ in S. Typhimurium and S. Agona.
1. The majority (84%) of ESBL/AmpC-producing *Salmonella* from humans were highly multidrug resistant (5-8 antibiotics).
2. No carbapenemase-producing *Enterobacteriaceae* were detected in livestock.
3. *E. coli* carrying $bla_{\text{OXA-48}}$ was detected in dog faeces in the Netherlands for the first time.
4. Colistin resistance gene *mcr-1* was identified at low-level in *E. coli* from livestock (1.2%) and at higher levels in retail meat from chicken (7.7%), but not in *Salmonella*.

4.1 ESBL/AmpC-producing bacteria

4.1.1 Randomly isolated ESBL/AmpC-producing bacteria from livestock in 2017

Surveillance of resistance to extended spectrum cephalosporins in the Netherlands is routinely done by random isolation of a minimum of 170 *Escherichia coli*, each representing one epidemiological unit, from faecal samples of food-producing animals, as prescribed by EFSA guidelines.[2] Isolates are tested for susceptibility to cefotaxime and ceftazidime and reduced susceptible isolates are determined based on EUCAST epidemiological cut-off values, as described in Chapter 3. Since 1998, cefotaxime resistance is observed at low levels in all animal species. Figure ESBL01 shows the percentage of cefotaxime resistant *E. coli* randomly picked from non-selective media derived from broilers, slaughter pigs (1998 – 2017), veal calves and dairy cows (2005 – 2017). In broilers, after 2003 an apparent increase in cefotaxime resistance was observed up to levels that varied between 15 – 20%, with the highest peak observed in 2007. The strong decline observed in 2011, from 18.3% to 8.1%, was most likely due to decreased usage of antibiotics since the spring of 2010 when the (off label) use of ceftiofur was ceased at Dutch hatcheries. A continuous low proportion of ESBL/AmpC-producing *E. coli* in broilers was observed in the last five years (<3%) and this was confirmed by the low level of cefotaxime resistance in 2017 (1.7%).

From a total of 1194 randomly selected *E. coli* isolates tested in 2017, five isolates from broilers and one isolate from pig (Table ESBL01) displayed reduced susceptibility (MIC > 0.25 mg/L) to cefotaxime (see also 3.2.1). No ESBL/AmpC-suspected *E. coli* isolates were found in veal calves and dairy cattle. Cefotaxime resistant isolates were screened for beta-lactamase gene families using an in-house developed RT-PCR (Geurts *et al,* 2017) or the Check-Points CT101 miniaturised micro-array (Check-Point, Wageningen, NL). Genes were confirmed by dedicated PCR and sequencing. All isolates with a negative array result for ESBL or AmpC genes were examined for promoter mutations in the chromosomal *ampC* genes. The results of this molecular typing are displayed in Table ESBL01.
Three different plasmid mediated ESBL/AmpC gene types were detected in the isolates from broilers: $bla_{\text{CTX-M-1}}$ (n=2), $bla_{\text{TEM-52c}}$ (n=1), and $bla_{\text{SHV-2}}$ (n=2). After two years of absence (2015-2016), $bla_{\text{TEM-52c}}$ was again detected as well as $bla_{\text{SHV-2}}$ missing since 2011 in broilers. The *E. coli* isolate from slaughter pig exhibited an atypical resistance pattern with borderline resistance to both cefotaxime and ceftazidime, but susceptibility to ampicillin. No ESBL/AmpC gene or chromosomal mutation were identified. For this reason the slaughter pig isolate was not considered to be an ESBL/AmpC-producer and therefore was not included in Table ESBL01 and Figure ESBL01. In general, no chromosomal *ampC* genes or $bla_{\text{CMY-2}}$ were detected in 2017. It can be concluded that by random isolation, only five cefotaxime resistant isolates (0.4%) associated to three plasmid mediated ESBL/AmpC genes were found in 2017, keeping the resistance proportion of the last three years under 1%. This confirms the major improvement compared to 2009 when ESBL/AmpC-producing isolates added up to 7.6%, before antibiotic usage reduction started in Dutch livestock.

[2] Report from the Task Force on Zoonoses Data Collection including guidance for harmonized monitoring and reporting of antimicrobial resistance in commensal Escherichia coli and Enterococcus spp. from food animals. http://www.efsa.europa.eu/en/efsajournal/pub/141r.htm.

Figure ESBL01 Trends in cefotaxime resistance (%) of *E. coli* randomly isolated from faeces of broilers, slaughter pigs, veal calves and dairy cows.

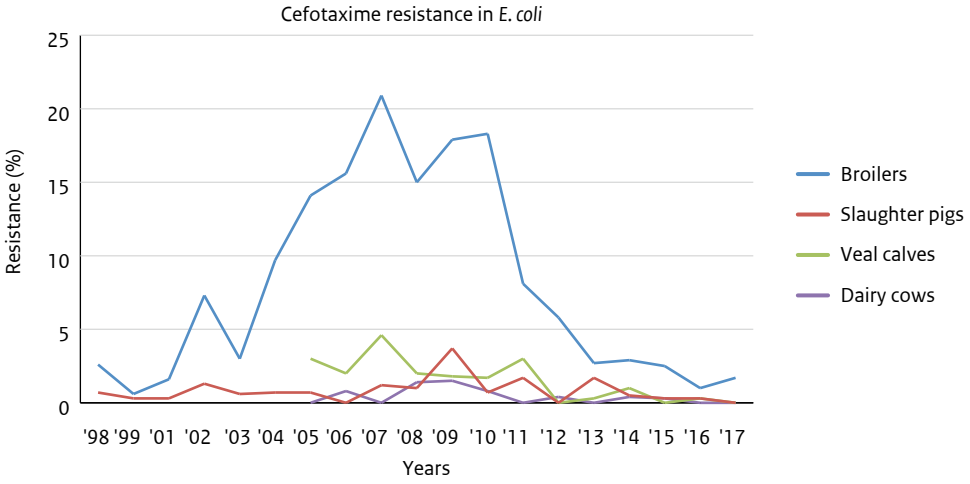


Table ESBL01 ESBL-genes found in *E. coli* isolates with reduced susceptibility to cefotaxime derived from broilers, veal calves, slaughter pigs, dairy cows and turkey (only 2011 and 2012) during 2007-2017.

	ESBLs isolated from						ESBL-genes detected											
Year	Broilers[c]	Veal calves	Slaughter pigs	Dairy cows[d]	Turkeys	Total ESBL suspected (n)	CTX-M-1-group[a]	CTX-M-2	CTX-M-9-group!	TEM-52c	TEM-20	SHV-12[b]	SHV-2	CMY-2	chromosomal ampC	no gene found	Total *E.coli* (n)	% ESBL of total *E. coli*
2007	9	6	2	0	n.t.	17	3	1		3				1	2	7	539	3,2
2008	66	4	3	2	n.t.	75	38	5	1	9			2	12	3	5	1026	7,3
2009	53	2	11	2	n.t.	68	34	7		2	1	8	1	12	3		894	7,6
2010	52	3	2	2	n.t.	59	21	6		5	1	9	4	5	3	5	1002	5,9
2011	23	5	5	0	6	39	9			8		9	2	3	3	5	1096	3,6
2012	26	2	0	1	n.t.	29	8			4		8		5		4	1328	2,2
2013	13	1	4	0	n.t.	18	7			4		3		3	1		1371	1,3
2014	11	3	2	0	n.t.	16	8			1		4			1	2	1519	1,1
2015	10	0	1	1	n.t.	12	3		2	1		1		2	3		1283	0,9
2016	3	1	1	0	n.t.	5	2			1				1	1		1492	0,3
2017	5	0	0	0	n.t.	5	2			1			2				1194	0,4
Total	271	27	31	8	6	343	135	19	3	39	2	42	11	44	20	28		

a. All were $bla_{CTX-M-1}$, only in 2011 one $bla_{CTX-M-3}$ gene was found in an isolate from a veal calf.
b. One combination of bla_{SHV-12} together with bla_{TEM-52} occured in 2012 in one broiler isolate.
c. In broilers, three combinations were found: in 2008: $bla_{CTX-M-1}$ with $bla_{CTX-M-2;}$ in 2009: $bla_{CTX-M-1}$ with bla_{SHV-12} and $bla_{CTX-M-1}$ with bla_{SHV-12} and bla_{CMY-2}.
d. In dairy cows, one combination of bla_{CMY-42} with $bla_{TEM-190}$.
n.t. : not tested

4.1.2 Selective isolation of ESBLs in 2017

As of 2014, selective culturing for ESBL/AmpC-producers in broilers was implemented together with the ongoing active surveillance in slaughter pigs and veal calves started in 2011. In 2017, caecal samples were taken at slaughter (white and rosé veal calves, slaughter pigs and broilers) and faecal samples at farms (dairy cows). Screening was done by overnight incubation of faecal samples (1 gram) in 9 ml Buffered Peptone Water (BPW) followed by selective isolation on MacConkey agar with 1 mg/L cefotaxime, according to EURL-AR protocols: http://www.eurl-ar.eu/233-protocols.htm.
Selective culturing for ESBL/AmpC-producers of meat samples was also implemented as of 2014. Meat samples (25 gram) were pre-enriched in 225 ml BPW followed by selective isolation on MacConkey agar with 1 mg/L cefotaxime and on Brilliance ESBL Agar (Oxoid, part of Thermo Fischer Scientific). From each plate, colonies with typical *E. coli* morphology were selected for bacterial species identification and confirmed *E. coli* isolates were screened for the identification of beta-lactamase gene families, as described above.

Results of selective isolation of ESBL/AmpC-producing *E. coli* in faeces

In 2017, 1203 faecal samples were screened for the presence of ESBL/AmpC-producing *E. coli*, each sample representing one slaughter batch of animals from one farm. Suspected ESBL/AmpC isolates comprised all *E. coli* growing on MacConkey with 1 mg/L cefotaxime including isolates carrying mutations in the chromosomal *ampC* gene promoter. Confirmed ESBL isolates encoding ESBL or AmpC genes, most likely located on a horizontally transmissible plasmid, are reported in Table ESBL02.
Of 1203 analysed samples, 22.6% were positive for ESBL/AmpC-producing *E. coli*, mainly due to the high prevalence in veal calves (36.7%, combining white and rosé). The increase already observed in 2016 in both white and rosé veal calves was confirmed in 2017 (40.5% and 28.3%, respectively), recording for the first time a higher prevalence in veal calves than in broilers (32.6%). As noted in the past, prevalence in white veal calves was higher than in rosé veal calves.
ESBL/AmpC-producing *E. coli* prevalence in broilers registered further decrease in 2017 compared to 2015-2016 (from 56.5-50.3% to 32.6%). The prevalence in slaughter pigs and in dairy cows decreased and stabilized at around 10%. In conclusion, 2017 marked an inversion in ESBL/AmpC-producing *E. coli* carriership in livestock, with increased prevalence in veal calves versus a reduction in broilers (Figure ESBL02). An explanation for this phenomenon is not available yet.

Figure ESBL02 Trends in cefotaxime resistance (%) of *E. coli* randomly isolated from faeces of broilers, slaughter pigs, veal calves and dairy cows.

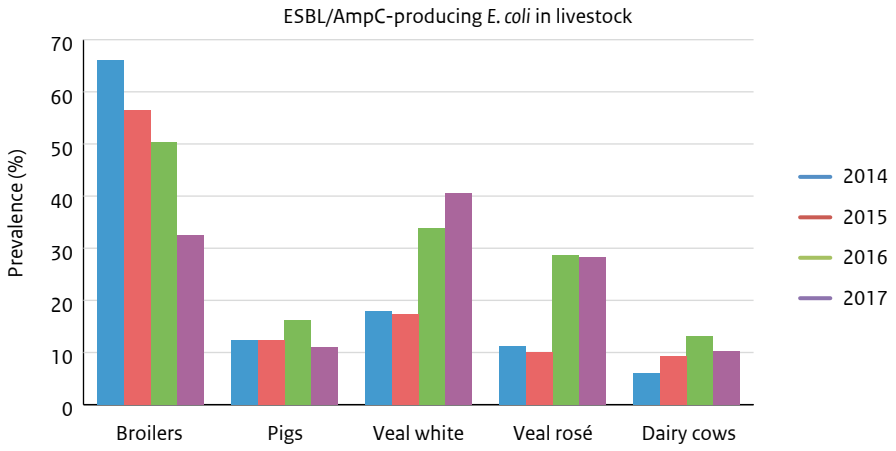


Table ESBL02 Prevalence of *E. coli* isolates showing reduced susceptibilty to cefotaxime derived from selective culturing of faecal samples from broilers, layers, ducks, slaughter pigs, veal calves and dairy cows taken at slaughter in 2017.

	N samples	N suspected ESBL	N confirmed ESBL	Prevalence(%) ESBL confirmed
Broilers	301	98	98	32.6
Pigs	300	47	33	11.0
Veal calves				
white	210	86	85	40.5
rosé	92	28	26	28.3
Dairy cows	300	36	30	10.0
Total	1203	295	272	22.6

ESBL/AmpC genes detected in animal faeces are reported in Table ESBL03. Comparable to former years (MARAN 2015, 2016 and 2017), high ESBL gene type variability was confirmed in 2017. $bla_{CTX\text{-}M\text{-}1}$ was still the dominant gene variant in all animal species (126 out of 295 genes detected), followed by $bla_{CTX\text{-}M\text{-}15}$ (n=46), $bla_{CMY\text{-}2}$ (n=28), $bla_{SHV\text{-}12}$ (n=20) and $bla_{TEM\text{-}52c}$ (n=14). The increased ESBL/AmpC-producing *E. coli* prevalence observed in veal calves was associated with the highest gene variability (12 different ESBL genes, mostly in white veal calves), comparable to the results of 2016. For the first time, prevalence of $bla_{CTX\text{-}M\text{-}15}$ (typically associated with humans) was higher than $bla_{CMY\text{-}2}$ (15.6% vs 9.5%, respectively), with a dominant distribution of $bla_{CTX\text{-}M\text{-}15}$ in cattle (veal calves and dairy cows) and $bla_{CMY\text{-}2}$ in broilers (Table ESBL03). Genes $bla_{CTX\text{-}M\text{-}9}$ and $bla_{CTX\text{-}M\text{-}14}$, also frequently associated with human isolates, were mostly detected in veal calves (with the exception of three $bla_{CTX\text{-}M\text{-}14}$ in slaughter pigs and one $bla_{CTX\text{-}M\text{-}14}$ in dairy cattle) comparable to the prevalence observed in 2016. Slaughter pig and dairy cow isolates didn't show significant differences compared to previous years. Chromosomal *ampC* types confirmed their role in conferring cefotaxime resistance as already observed in 2015-2016 with relatively high numbers in slaughter pig and dairy cow isolates (29.8% and 16.7%, respectively). As in previous years, no combination of ESBL genes was detected with the exception of one *E. coli* isolate from dairy cow exhibiting the AmpC beta-lactamase $bla_{CMY\text{-}42}$ gene together with beta-lactamase gene $bla_{TEM\text{-}190}$.

Results of selective isolation of ESBL/AmpC-producing *E. coli* in raw meat

Prevalence of ESBL suspected isolates in fresh raw meat was investigated and results are shown in Table ESBL04. Meat preparations (except for imported frozen poultry meat with approximately 1% salt) were not screened in 2017. Out of 1626 fresh meat samples, 156 were confirmed positive for ESBL/AmpC-producing *E. coli* (9.6%), with the highest prevalence in imported chicken meat (56.1%). This result was comparable to previous years (67-61%, 2014-2016) and in line with the decreasing prevalence started in 2012 (83%). Fresh turkey meat registered a prevalence of 17,6%, comparable to the reduction observed in fresh turkey meat in recent years, from 50.9% in 2014. While beef showed a reduction compared to 2016 (from 2.0% to 0.9%), a slightly higher ESBL/AmpC prevalence was observed in lamb (from 2.7% to 5.1%) and veal (from 4.4% to 7.5%) compared to 2016. ESBL/AmpC-producing *E. coli* prevalence in veal has been increasing from 2014 onwards (from 0% to 3.8% in 2014 and 2015, respectively). Yet, for some types of meat the number of samples was relatively low, so the figures should be interpreted with care.

All 156 isolates were confirmed by MALDI-TOF as *E. coli* and molecularly characterized. Table ESBL05 shows the different ESBL/AmpC gene types detected in meat. Most of the genes found in beef and veal ($bla_{CTX\text{-}M\text{-}1}$, $bla_{CTX\text{-}M\text{-}15}$, $bla_{CTX\text{-}M\text{-}32}$ and $bla_{CTX\text{-}M\text{-}55}$) were also present in faecal samples of veal calves indicating possible faecal contamination during slaughter and/or meat processing. Chicken meat displayed more ESBL/AmpC gene variability than Dutch broiler faecal samples, with $bla_{CTX\text{-}M\text{-}2}$, $bla_{CTX\text{-}M\text{-}8}$, $bla_{CTX\text{-}M\text{-}9}$ and $bla_{SHV\text{-}2}$ not detected in the latter. This difference in gene variation might be explained by the fact that fresh retail chicken meat in Dutch supermarkets is not exclusively produced in the Netherlands, but can also originate from neighbouring countries within the EU. Moreover, the inclusion of imported frozen chicken meat from outside the EU (mainly from South America and Asia) inevitably increases the variability of ESBL/AmpC genes detected. Coexistence of different ESBL genes was observed in two *E. coli* isolates from chicken meat: $bla_{CTX\text{-}M\text{-}2}$ with $bla_{CTX\text{-}M\text{-}8}$, and $bla_{CMY\text{-}2}$ with $bla_{TEM\text{-}52c}$. The dominant human associated $bla_{CTX\text{-}M\text{-}15}$ gene was detected in higher prevalence than 2015-2016 (from 5.6-5.7% to 11.5%) in almost all meat types, with the highest prevalence observed in fish and shrimps (88%) and veal (29%), in line with the prevalence observed in veal calf faecal samples (Table ESBL03). Other frequent gene

types were bla_{CMY-2} and bla_{SHV-12} typically found in broiler faecal samples too. Chromosomal *ampC* types were detected mainly in *E. coli* isolates from lamb meat.

Table ESBL03 Beta-lactamases identified in *E. coli* from broilers, slaughter pigs, veal calves, and dairy cows in 2017. Data derived from the active surveillance of ESBL-producing *E. coli* at slaughter.

		Broilers	Slaughter pigs	Veal calves White	Veal calves Rose	Dairy cows	Total
CTX-M-1 group							
	CTX-M-1	46	23	35	13	9	126
	CTX-M-3		1			1	2
	CTX-M-15			25	9	12	46
	CTX-M-32	1		5	1		7
	CTX-M-55	1		2			3
CTX-M-2 group							
	CTX-M-2			1		1	2
CTX-M-8/25 group							
	CTX-M-8					1	1
CTX-M-9group							
	CTX-M-9			1			1
	CTX-M-14		3	5		1	9
	CTX-M-65			3	1	3	7
TEM							
	TEM-52c	6	3	4	1		14
	TEM-52cVar	4	2				6
	TEM-225						1
SHV							
	SHV-12	15	1	3		1	20
CMY							
	CMY-2	25		1	1	1	28
	CMY-42/TEM-190					1	1
Chromosomal ampC							
	ampC-type-3		14	1	2	5	22
	ampC-type-3-like						
Total		98	47	86	28	36	295

Table ESBL04 Prevalence of ESBL/AmpC-positive *E. coli* isolates from raw meat products in the Netherlands in 2017.

Animal source		N screened	N ESBL/AmpC positive (confirmed)	% ESBL/AmpC positive
Beef				
	fresh meat	528	5	0,9
Veal				
	fresh meat	226	17	7,5
Pork				
	fresh meat	275	4	1,5
Chicken				
	fresh meat [a]	228	72	31,6
	import [b]	57	32	56,1
Turkey				
	fresh meat [c]	38	6	15,8
	import [d]	4	1	25,0
Lamb				
	fresh meat	198	10	5,1
Sheep				
	fresh meat	4	0	0,0
Goat				
	fresh meat	8	1	12,5
Fish and shrimps				
	fresh meat	56	7	12,5
Crocodile				
	fresh meat	3	1	33,3
Frog				
	fresh meat	5	1	20,0
Total		1630	157	9,6

a. Fresh broiler retail meat originates from animals produced within EU (mainly, but not exclusively from the Netherlands)

b. Imported frozen meat preparations originates from countries outside EU (mainly from South America or Asia)

c. Fresh turkey retail meat originates from animals produced within EU (but often not from the Netherlands)

d. Imported frozen turkey meat preparations originates from countries outside EU (mainly from South America or Asia)

Table ESBL05 Beta-lactamases identified in *E. coli* from raw meat products in the Netherlands in 2017.

ESBL gene		Chicken	Turkey	Beef	Veal	Pig	Lamb	Goat	Crocodile	Frog	Fish and shrimpls	Totaal
CTX-M-1 group												
	CTX-M-1	20	4	2	3	1	1					31
	CTX-M-15	1	1	1	5	1	2			1	6	18
	CTX-M-32	1			4							5
	CTX-M-55	2		1	2						1	6
CTX-M-2 group												
	CTX-M-2	9		1	1							11
	CTX-M-2/ CTX-M-8	1										1
CTX-M-8/25 group												
	CTX-M-8	4	1									5
CTX-M-9 group												
	CTX-M-14						2					2
	CTX-M-65				1							1
TEM												
	TEM-52	1			1							2
	TEM-52c	4										4
	TEM-52cVar	5										5
SHV												
	SHV-12	14					1	1				16
	SHV-2	1										1
	SHV-2a	1										1
CMY												
	CMY-2	38				1			1			40
	CMY-2/TEM-52c	1					1					2
Chromosomal ampC												
	ampC-type-3					1	3					4
	ampC-type-11	1										1
Total		104	6	5	17	4	10	1	1	1	7	156

ESBL/AmpC-producing Salmonella

Surveillance of resistance to extended spectrum cephalosporins is also routinely performed in *Salmonella enterica* isolated in the Netherlands from both human and animal (meat) source.

In 2017, the NVWA tested 187 *Salmonella* isolates mostly originating from imported meat samples, also including imported seafood and herbs. A high proportion of the isolates exhibited resistance to cefotaxime (n=69) in four different serovars: Heidelberg (=65), Minnesota (n=2), Abony (n=1), and Schwarzengrund (n=1), exclusively originating from imported chicken meat from outside the EU. No further molecular characterization was performed on these cefotaxime resistant *Salmonella* isolates from meat.

A total of 1697 *Salmonella* of human origin isolated in 2017 were sent by RIVM to WBVR to be tested for susceptibility to cefotaxime and ceftazidime. In total, 31 cefotaxime or ceftazidime resistant *Salmonella* were identified (Table ESBL06). The prevalence of ESBL/AmpC-producing *Salmonella* was 1.8%, comparable to that observed in 2015 and 2016 (1.9% and 1.7%, perspectively) and almost half of 2013 (4%). The predominance of *S.* Kentucky observed in 2016 was confirmed in 2017 (n=18), followed by Typhimurium (n=8), and four other serovars identified to carry ESBL/AmpC genes (material and methods are the same as described for *E. coli* in the previous section).

ESBL/AmpC genes detected in *Salmonella* of human origin are reported in Table ESBL06. The most represented gene types were: i) $bla_{\text{CTX-M-14b}}$, generally associated with *S.* Kentucky; ii) $bla_{\text{CTX-M-9}}$ in *S.* Typhimurium; and iii) $bla_{\text{CMY-2}}$ in *S.* Typhimurium and *S.* Agona. Comparable to previous years' results, prevalence of $bla_{\text{CMY-2}}$ in 2017 was assessed at 10%. Similarly, $bla_{\text{CTX-M-1}}$, $bla_{\text{CTX-M-15}}$ and $bla_{\text{CTX-M-55}}$ were less represented than previous years. $bla_{\text{CTX-M-9}}$ and $bla_{\text{CTX-M-14b}}$ confirmed their predominant role compared to previous years with an increase from 3-8% (2016) to 19-52% (2017), respectively. No ESBL/AmpC gene combination was detected in *Salmonella*.

All cefotaxime resistant *Salmonella* isolates of human origin were highly multidrug resistant, as shown in Table ESBL07. The increased multi-resistance observed in the last years compared to 2014 (23%) was similar to that of 2017 with most of the isolates resistant to 5-8 antibiotics (84%). 3% of the isolates were resistant to 9 out of 10 antibiotics but no resistance was detected against meropenem. For the first time one *Salmonella* isolate was resistant to azithromycin. As for 2016, colistin resistance was 0% in 2017, compared to 8.8% in 2015.

ESBL/AmpC gene types found in *Salmonella* since 2007 are summarized in Table ESBL08. Every year $bla_{\text{CMY-2}}$ and genes belonging to the $bla_{\text{CTX-M-1}}$ -group have been associated to *Salmonella* isolates from diverse sources. After detection in 2015, $bla_{\text{CTX-M-2}}$ was not detected in 2016 and in 2017. The most prevalent group was $bla_{\text{CTX-M-9}}$ (23 out of 31 genes) confirming the increase registered in the last three years. $bla_{\text{DHA-1}}$ was identified for the first time in 2016 in a human isolate of *S.* Bovismorbificans and in *S.* Kentucky in 2017. Overall, *Salmonella* isolates showed different ESBL/AmpC gene variability than in 2016.

In conclusion, ESBL/AmpC-producing *E. coli* are common in Dutch food-producing animals and in raw meat mainly of poultry origin. ESBL/AmpC genes were detected in 0.4% of randomly isolated *E. coli*. For the first time selective culturing of livestock faecal samples showed higher prevalence in veal calves than in broilers (36.7% vs 32.6%, respectively). The dominant ESBL/AmpC gene type was confirmed to be $bla_{\text{CTX-M-1}}$ in all animal species. The human ESBL gene $bla_{\text{CTX-M-15}}$ was frequently found in veal calf and dairy cow faecal samples and derived meat products, and only rarely found in broilers and chicken products confirming the observations of 2015-2016. Human *Salmonella* isolates were mostly associated with $bla_{\text{CTX-M-9}}$ -group genes and showed a multidrug resistant phenotype.

Table ESBL06 Beta-lactamases in *Salmonella* isolated from humans in 2017.

	CTX-M-1 group			CTX-M-9 group			SHV	CMY	DHA	
Serovar	**CTX-M-1**	**CTX-M-15**	**CTX-M-55**	**CTX-M-9**	**CTX-M-14b**	**CTX-M-65**	**SHV-12**	**CMY-2**	**DHA-1**	**Total**
1,4,5,12:i:-			1							1
Agona								1		1
Infantis	1					1				2
Kentucky		1			16				1	18
Typhimurium				6				2		8
Virchow							1			1
Total	**1**	**1**	**1**	**6**	**16**	**1**	**1**	**3**	**1**	**31**

Table ESBL07 Resistance and multidrug resistance percentages of human ESBL-producing *Salmonella* in the Netherlands in 2017.

Antimicrobials	R%
Ampicillin	100,0
Cefotaxime	100,0
Ceftazidime	77,4
Gentamicin	80,6
Tetracycline	96,8
Sulfamethoxazole	90,3
Trimethoprim	16,1
Ciprofloxacin	93,5
Nalidixic acid	64,5
Chloramphenicol	35,5
Azithromycin	3,2
Colistin	0,0
Meropenem	0,0
Tigecycline	19,4

Multi drug resistance	N = 31
0	0%
1	0%
2	0%
3	3%
4	10%
5	3%
6	29%
7	45%
8	6%
9	3%
10	0%

Table ESBL08 ESBL-genes found in *Salmonella* isolates displaying reduced susceptibility to cefotaxime during 2007-2017.

Year	CTX-M-1-group[a]	CTX-M-2[b]	CTX-M-8	CTX-M-9-group[c]	TEM-52	TEM-20	SHV-12[d]	CMY-2[e]	ACC-1	DHA-1	Total ESBL	Total *Salmonella* tested	% ESBL of total *Salmonella*
2007	9	13			17	2	4	2			47	1514	3,1
2008	25	12	1	1	13	1		6	2		61	2149	2,8
2009	12	4		2	3		1	9			31	2232	1,4
2010	8	3		1	2		3	4			21	1715	1,2
2011	5	3		1	1		2	13			25	1444	1,7
2012	14	5		2	2			10	1		34	1795	1,9
2013	1	3	5	4	5	1		36			55	1369	4,0
2014	6		2	3	1			21			33	1688	2,0
2015	13	2		6	1			12			34	1761	1,9
2016[f]	7			15	2			10		1	36	2117	1,7
2017[g]	3			23			1	3		1	31	1697	1,8
Total	103	45	8	58	47	4	11	126	3	2	408	19481	2,1

a. *contains $bla_{CTX-M-1}$ (n=70, in all years), $bla_{CTX-M-55}$ (n=8, 2008-2010,2012, 2015), $bla_{CTX-M-15}$ (n=10, 2011-2013), $bla_{CTX-M-3}$ (n=3 , 2010, 2012) and a combination with bla_{CMY-2} (n=2, 2014, 2015).*

b. *In 2008 one combination of $bla_{CTX-M-2}$ with bla_{TEM-52} was found in S. Paratyphi B var Java.*

c. *contains $bla_{CTX-M-9}$ (n=8, 2008-2009, 2012-2015), $bla_{CTX-M-14}$ (n=6, 2009-2012, 2015) and $bla_{CTX-M-65}$ (n=6, 2013-2015).*

d. *In 2007 three S. Concord were found containing both bla_{SHV-12} and $bla_{CTX-M-15}$.*

e. *In 2015 a combination of bla_{CMY-2} and bla_{TEM-52} was found in S. Oranienburg and a combination of bla_{CMY-2} with $bla_{CTX-M-1}$ in S. Molade*

f. *In 2016, one S. Minnesota isolate obtained from poultry meat at NVWA was not included in the molecular analysis.*

g. *In 2017 only human isolates were molecularly characterised.*

4.2 Carbapenemase producing Enterobacteriaceae

4.2.1 Monitoring in livestock

In 2015 a sensitive molecular method was applied to screen for carbapenemase producers, extended spectrum beta-lactamases that can also hydrolyse carbapenems (MARAN 2016 for method details). This is important in an environment with a very low anticipated prevalence of carbapenem resistance. All faecal samples sent by NVWA to WBVR for antimicrobial resistance surveillance were screened with this method. Samples were grown overnight in BPW and after incubation the culture was centrifuged and DNA isolated from pellet. Five individual samples were pooled and analysed together. A commercial RT-PCR (Check-Points, CarbaCheck MDR RT) that can detect the most important carbapenemase gene families (bla$_{KPC}$, *bla*$_{NDM}$, *bla*$_{VIM}$, *bla*$_{IMP}$ and *bla*$_{OXA-48}$) was used according to manufacturer's instructions. If RT-PCR gave suspicious or positive results, a step-wise analysis was performed to confirm the results:

1. RT-PCR was performed on purified DNA of the 5 individual samples of the pool;
2. If PCR was positive, genes were identified with Sanger sequencing;
3. Original faecal sample and corresponding broth culture of suspected positive samples were inoculated for bacterial isolation on commercial selective plates (ChromID CARBA and ChromID OXA, Biomerieux, for *Enterobacteriaceae*) and on HIS plates with 0.125 mg/L ertapenem (for *Shewanella* spp).

Carbapenemase screening in 2017 (n=1200) resulted in six *bla*$_{OXA-48}$ -like positive faecal samples in the RT-PCR (three broilers, two slaughter pigs and one dairy cow). *bla*$_{OXA-48}$ -like genes are known to be chromosomally associated with *Shewanella* spp. In three samples the presence of *bla*$_{OXA-48}$ -carrying *Shewanella* was confirmed by bacterial culturing followed by PCR and sequencing: *bla*$_{OXA-48b}$ (n=2) and *bla*$_{OXA-199}$ (n=1). These results confirm the findings of previous years, as no carbapenemase-producing *Enterobacteriaceae* were isolated from livestock in the Netherlands. *bla*$_{OXA-48}$ -like genes have also been found in faecal samples in 2013, 2014, 2015 and 2016 (MARAN 2016 and 2017). Given the role of *Shewanella* spp. as natural progenitor of this carbapenemase family (Zong, 2012), these genes were considered of environmental origin and not a public health risk. Screening for carbapenemase-producing isolates in faecal samples of food-producing animals will continue in 2018.

4.2.2 Monitoring in companion animals

The prevalence of carbapenemase producing *Enterobacteriaceae* (CPE) in companion animals in Europe is relatively low. CPE have been observed in pet dogs from Germany (Stolle *et al,* 2013), Spain (González-Torralba *et al,* 2016) and France (Melo, *et al,* 2017). Monitoring to detect introduction of CPE in companion animals in the Netherlands was initiated in 2015. The screening for CPE comprised an initial retrospective study and a prospective study. Until 2016, CPE have not been detected in the Netherlands (MARAN 2017). The prospective study was continued in 2017.

Fecal samples of cats and dogs were obtained through the Veterinary Microbiological Diagnostic Center (VMDC) of Utrecht University. Because the expected prevalence of CPE is low and reported CPE are frequently multi-resistant, the inclusion criterion for dog fecal samples was antibiotic treatment of the animal. Since cats are not frequently treated with antimicrobials, no inclusion criterion was defined and all available fecal samples from cats submitted to VMDC were included in the study. In 2017, 200 fecal samples from cats and 138 fecal samples from dogs were screened. From each sample, 0.5 gram feces was suspended in 4.5 ml TSB broth, supplemented with 50 mg/L vancomycin for enrichment. The suspension was directly inoculated on ChromID Carba-Smart agar plates (BioMerieux). Both the Smart Agar and the enrichment broth were cultured overnight at 37°C. After enrichment, the broth was again inoculated and cultured on ChromID Carba-Smart agar (BioMerieux). In addition, total DNA of the enrichment broth was isolated for molecular screening by PCR for the targets bla_{NDM} (Manchanda *et al,* 2011), bla_{KPC} (Bradford *et al,* 2004), bla_{IMP} (Ellington *et al,* 2007), bla_{VIM} (Ellington *et al,* 2007), bla_{OXA} -groups 23, 24, 51 and 58. (Voets *et al,* 2011) and bla_{OXA} -group-48 (Poirel *et al,* 2004).

In 2017, all fecal samples from cats were negative for CPE. One isolate from dog was positive for *E. coli* harboring bla_{OXA-48}. This was the first CPE isolated from a live animal in the Netherlands. The sample was submitted to the diagnostic laboratory of the veterinary faculty (VMDC) for parasitological diagnostics because of chronic diarrhea and the dog had been treated with metronidazole for 10 days. Molecular analysis of the isolate is ongoing but preliminary analysis suggests that the bla_{OXA-48} gene is transferable because located on a mobile element (J. Hordijk, personal communication).

4.2.3 Monitoring in imported seafood

In 2017, 56 batches of frozen fish and shrimps originating from fish farms in South-East Asia were screened for the presence of CPE. Two isolates of carbapenemase-producing *Enterobacter cloacae* complex were detected in different batches of frozen shrimps, both exhibiting resistance to carbapenems but not to third generation cephalosporins. The first isolate originated from India (April 2017) and preliminary analysis suggests the presence of a new plasmid located carbapenemase gene (M. Brouwer, personal communication). The second isolate originated from Vietnam (August 2017) and harboured a chromosomally located bla_{IMI-1} embedded in an insertion element (EcloIMEX) (Brouwer *et al,* 2018).
Consumption of antimicrobials is high in South-East Asia both in humans and in animals, and aquaculture represents an environment with high selective pressure for resistant bacteria, including CPE and potential for fecal contamination. Therefore detection of CPE in imported food products from this area is not surprising.

4.3 Colistin resistance

As published in MARAN 2016 a retrospective study revealed the low prevalence of the colistin resistance gene *mcr-1* in *E. coli* from livestock (≤ 1%) and meat (2%), and in *Salmonella* from poultry meat (1%) in the period 2010 – 2015. The fact that no *mcr-1* genes were identified in randomly isolated indicator *E. coli* from faecal samples from 2014 and 2015 suggests a decreasing trend in the occurrence of this gene. Like in former years, no colistin resistant isolates were identified amongst the randomly selected indicator *E. coli* isolated from faecal samples in 2017.

To gain more knowledge on the current spread of *mcr-1* and its allelic variants in livestock, selective monitoring was started in 2016 and continued in 2017 as part of the national surveillance program on antibiotic resistance in animals. In order to increase the sensitivity of the test, selective enrichment was started in 2017 by using BPW broth with 2 mg/L colistin. Purified DNA of pooled PBW cultures (five samples per pool) from a total of 1200 faecal samples were tested with conventional PCR for the presence of *mcr-1* and *mcr-2* according to EURL-AR protocols (http://www.eurl-ar.eu/233-protocols.htm). In case of a PCR positive pool, individual samples were tested followed by direct culturing of the original BPW broth on MacConkey agar with 2 mg/L colistin. As a result, *mcr-1* positive *E. coli* were identified in fourteen faecal samples (1.2%) from selective culturing in several animal species: veal calves (n=9, 3.0%), broilers (n=3, 1.0%) and slaughter pigs (n=2, 0.7%). Despite an increased test sensitivity, the proportion of *mcr*-positive faecal samples remained low.
In retail meat three randomly isolated colistin resistant *E. coli* [chicken (n=2) and turkey meat (n=1)] were confirmed as *mcr-1* carriers which is indicative for a higher prevalence in poultry meat than in broilers. BPW enrichment from fresh chicken meat indicated 7.7% of the samples (3/39) was positive for *mcr-1* by PCR screening. The higher prevalence in chicken meat compared to faecal broiler samples can be explained by the fact that part of fresh retail meat in Dutch supermarkets originates from other countries where use of colistin in livestock might be the reason for higher *mcr* prevalence in meat. Finally, *mcr-1* was not identified in *Salmonella*.
In summary, *mcr-1* was identified at low-level in *E. coli* from different livestock species and at higher levels in retail meat from chicken and turkey, but not in *Salmonella*.

References

Bradford *et al;* Emergence of carbapenem-resistant *Klebsiella* species possessing the class A carbapenem-hydrolyzing KPC-2 and inhibitor-resistant TEM-30 β-Lactamases in New York city. Clin Infect Dis. 2004 Jul 1;39(1):55-60

Brouwer M.S.M. *et al; Enterobacter cloacae* complex isolated from shrimps from Vietnam encoding bla_{IMI-1}, resistant to carbapenems but not cephalosporins. Antimicrob Agents Chemother. 2018, doi: 10.1128/AAC.00398-18.

Cohen-Stuart *et al;* Guideline for phenotypic screening and confirmation of carbapenemases in *Enterobacteriaceae*. Int J Antimicrob Agents, 2010: 36(3):205-10

Ellington *et al;* Multiplex PCR for rapid detection of genes encoding acquired metallo-beta-lactamases. J Antimicrob Chemother. 2007 Feb;59(2):321-2

Geurts Y. *et al*. Development of sensitive and cost-effective Real-time PCR assays for rapid detection of the beta-lactamase genes CTX-M-1, SHV, TEM and AmpC gene CMY-2 in *Enterobacteriaceae*. 2017, 27th ECCMID, Vienna (Austria).

González-Torralba *et al;* Survey of carbapenemase-producing *Enterobacteriaceae* in companion dogs in Madrid, Spain. Antimicrob Agents Chemother, 2016: 60(4):2499-2501

Manchanda *et al;* Development of TaqMan real-time polymerase chain reaction for the detection of the newly emerging form of carbapenem resistance gene in clinical isolates of *Escherichia coli*, *Klebsiella pneumoniae*, and *Acinetobacter baumannii*. Indian J Med Microbiol. 2011 Jul-Sep;29(3):249-53

MARAN Reports (2002-2017): https://www.wur.nl/nl/Expertises-Dienstverlening/Onderzoeksinstituten/Bioveterinary-Research/Publicaties/MARAN-Rapporten.htm

Melo *et al;* OXA-48-producing ST372 *Escherichia coli* in a French dog. Antimicrob Chemother. 2017; 72(4):1256-1258

Poirel *et al;* Emergence of oxacillinase-mediated resistance to imipenem in *Klebsiella pneumoniae*. Antimicrob Agents Chemother. 2004 Jan;48(1):15-22.

Stolle *et al;* Emergence of OXA-48 carbapenemase-producing *Escherichia coli* and *Klebsiella pneumoniae* in dogs. J Antimicrob Chemother, 2013; 68(12);2802-2808

Voets *et al;* A set of multiplex PCRs for genotypic detection of extended-spectrum β-lactamases, carbapenemases, plasmid-mediated AmpC β-lactamases and OXA β-lactamases. Int J Antimicrob Agents. 2011 Apr;37(4):356-9

Zong Z. Discovery of $bla_{OXA-199}$, a chromosome-based bla_{OXA-48} -like variant, in *Shewanella xiamenensis*. PLoS ONE 2012; 7: e48280.