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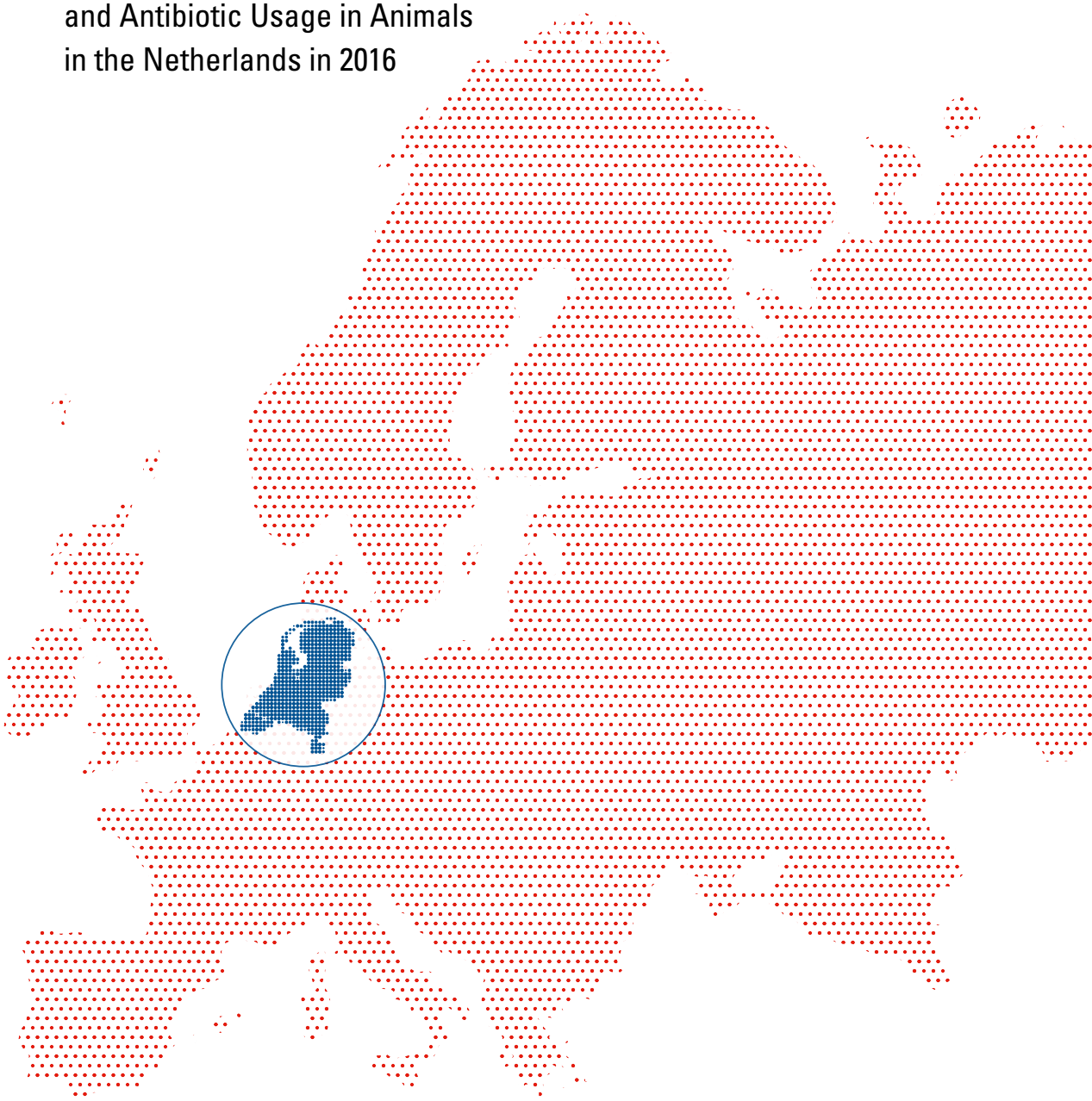
MARAN 2017

Monitoring of Antimicrobial Resistance and Antibiotic Usage in Animals in the Netherlands in 2016



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WAGENINGEN UNIVERSITY & RESEARCH

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Monitoring of Antimicrobial Resistance
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June 2017

Colophon

This report is published under the acronym MARAN-2017 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 Authority (SDa). The information presented in MARAN-2017 is based on total sales data and animal specific usage of antimicrobial agents in animal husbandry and the development of antimicrobial resistance in bacteria of animal origin and of relevance to public health.
MARAN-2017 is published in a combined back-to-back report with NETHMAP-2017. 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 websites of the Netherlands Veterinary Medicines Authority (www.autoriteitdiergeneesmiddelen.nl).
MARAN-2017 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 in 2016 (176 tonnes) showed a remarkable reduction (15%) compared to 2015 (206 tonnes). In relation to 2009, the index year used by the Ministry of Economic Affairs, in 2016 total sales decreased by 64.4%. Compared to 2007, the year with highest sales (565 tonnes), the decrease in sales is 69%. This year it was possible to allocate most sold antimicrobial veterinary medicinal products to the species the products are used in by using several alternative data sources. Most classes of antibiotics showed a decrease in sales in 2016, but some increased. In all but one sectors (veal calves, dairy cattle, pigs broilers and turkeys) a reduction in consumption was realized. In other cattle increased consumption is noted, albeit this consumption is low. The use of antibiotics of critical importance to human health care (especially cephalosporins of 3rd and 4th generation) is reduced to an absolute minimum.

Antimicrobial resistance

In 2016 *S.* Enteritidis (N = 438) followed by *S.* Typhimurium (N = 260) together with the monophasic variant of Typhimurium: 1,4,5,12:i:- (N = 229), were most frequently isolated from humans suffering from salmonellosis. In pigs, the monophasic variant of *S.* Typhimurium dominated. In cattle, besides *S.* Typhimurium variants, *S.* Dublin was most commonly isolated. In poultry (including poultry products, broilers and layers), the number of *S.* Paratyphi B var. Java further reduced in 2016. Also *S.* Infantis, still predominant in 2015, was less frequently isolated in 2016. The prevalence of *S.* Enteritidis increased compared to 2015 and was the most predominant serovar in poultry in 2016. Highest resistance levels were observed in *S.* Kentucky (travel related), monophasic *S.* Typhimurium 1,4,[5],12:i:-, other *S.* Typhimurium, *S.* Paratyphi B var. Java and to a lesser extent in *S.* Infantis and *S.* Newport. Ciprofloxacin resistance was most common amongst isolates from humans and poultry. Predominant serovars were *S.* Enteritidis (23%), *S.* Typhimurium (18%) and *S.* Kentucky (11%). In 2016, the total number of cefotaxime resistant (MIC > 0.5 mg/L) ESBL suspected *Salmonella* isolates was 28/1954 (1.4%), among nine different serovars, predominantly isolated from human sources. No carbapenemase producing *Salmonella* were found in 2016.

As a result of prioritization and changes in legislation, since 2014 the focus of the surveillance of antimicrobial resistance in *Campylobacter* is mainly in isolates from poultry (including broilers, laying hens and ducks) and poultry meat.
Resistance rates in *C. jejuni* from broilers was somewhat lower, whereas rates in poultry meat did not substantially change in 2016, compared to 2015. Overall, resistance levels were higher in *C. coli* than in *C. jejuni* isolates. Resistance rates for quinolones in *C. coli* isolates from broilers, laying hens and poultry meat decreased since 2015. Levels of resistance of *C. jejuni* for tetracycline and the quinolones were substantially higher in broilers than in ducks and laying hens. In *C. jejuni* from milk sheep and milk goats, resistance percentages were highest for ciprofloxacin, nalidixic acid and tetracycline, but at much lower levels than in poultry. Ciprofloxacin resistance in *Campylobacter* isolates from human patients is still high (with a slight decrease in 2016), 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 levels were higher for all three antimicrobials tested in travel related infections compared to domestically acquired campylobacteriosis.

After a tendency of increasing resistance to ampicillin, tetracycline, sulfamethoxazole and trimethoprim since 2009 in STEC O157 isolates from humans, in 2016, a decrease was found for ampicillin (from 14.3% to 10.7%), sulfamethoxazole (from 15,6% to 14.7%) and trimethoprim (from 14.3% to 8.0%). Resistance for the quinolones (ciprofloxacin and nalidixic acid) was not detected in human STEC O157 isolates.

In 2016, resistance levels of indicator *E. coli* from faecal samples showed a tendency to decrease in broilers and pigs and stabilized in veal calves and dairy cattle. In isolates from chicken meat resistance levels were substantially lower than in isolates from turkey meat. The levels of resistance were similar to 2015 in both types of poultry meat. Resistance levels for almost all tested antibiotics were much higher in samples of imported chicken and turkey meat than in samples from retail. Resistance to third-generation cephalosporins was low (< 1%) in all tested animal species. Although resistance to fluoroquinolones is decreasing, it was still commonly present in indicator *E. coli* from broilers and to a lesser extent in white veal calves, but substantially decreased in *E. coli* from white veal calves. Among indicator *E. coli* from animals and meat, resistance levels to ampicillin, tetracycline, sulphonamides and trimethoprim were still reasonably high in broilers, turkey, pigs and veal calves.
Levels of resistance in *E. coli* from rosé veal calves were substantially lower than those from white veal calves for almost all antibiotics tested.

Susceptibility testing of enterococci is considered of lesser priority than *E. coli*, also in the new EU legislation. Therefore, from 2016, no enterococci from faecal samples were tested, but in 2016 *Enterococcus faecalis* and *E. faecium* were isolated from chicken and turkey meat samples. The poultry meat samples were taken at retail.
In chicken meat, highest resistance levels were observed for erythromycin (55.4% for *E. faecalis* and 57.1% for *E. faecium*) and tetracycline (66.1% and 25.0% respectively). In addition, a high level of resistance was observed for quinu/dalfopristin in *E. faecium* (42.9%). In turkey meat, highest resistance levels were observed for erythromycin (65.1% for *E. faecalis* and 58.8% for *E. faecium*) and tetracycline (88.9% and 76.5% respectively). A high resistance percentage was also observed for quinu/dalfopristin in *E. faecium* (58.8%).

ESBL/AmpC-producing *Escherichia coli* represented 0.3% of the randomly isolated *E. coli*, the lowest proportion observed since 2007. In spite of the above, selective culturing in livestock faeces indicated that the prevalence (% of animal carriers) of ESBL/AmpC-producing *E. coli* marked a general tendency to increase in livestock, excluding broilers and layers. Currently an explanation for this phenomenon is lacking.
A follow up of the 2009 study on within-farm prevalence of ESBL/AmpC-producing *E. coli* in broilers showed a significant decrease from 66% in 2009 to 38% in 2016. The proportion of fresh chicken meat with ESBL/AmpC-producing *E. coli* isolates decreased to 24% (67% in 2014, 39.4% in 2015). In imported chicken meat the proportion was much higher with 61.2%. The most prevalent ESBL/AmpC gene in *E. coli* from livestock and meat was $bla_{\text{CTX-M-1}}$ in almost all animal species followed by $bla_{\text{CMY-2}}$, $bla_{\text{SHV-12}}$, $bla_{\text{TEM-52}}$ and $bla_{\text{CTX-M-14}}$.

The prevalence of ESBL/AmpC-producing *Salmonella* in 2016 was 1.7%, confirming the decreasing trend observed in the period 2013–2015. Most represented ESBL/AmpC genes were $bla_{\text{CMY-2}}$, generally associated with *S.* Saintpaul, $bla_{\text{CTX-M-14b}}$ in *S.* Kentucky, and $bla_{\text{CTX-M-9}}$ in *S.* Typhimurium. The majority of ESBL-producing *Salmonella* isolates from humans were highly multidrug resistant, with most of the isolates showing a resistant phenotype to 5-8 antibiotics (67%).

No carbapenemase-producing *Enterobacteriaceae* were detected in active surveillance in livestock. Only $bla_{\text{OXA-48}}$-like genes were detected in two faecal samples from veal calf and slaughter pig associated with *Shewanella* spp.. In a retrospective study in companion animals, horses and as well as in a prospective study in companion animals no carbapenemase-producing *Enterobacteriaceae* were detected.

The colistin resistance gene *mcr-1* was incidentally found in *E. coli* from livestock (0.5%). In retail meat *mcr-1* was most frequently identified in turkey (8.3%), but also in chicken (0.7%).

It can be concluded that the sales of antibiotics for animals further decreased in 2016. Moreover, in all but one sectors (veal valves, dairy cattle, pigs, broilers and turkeys) a reduction in consumption was realized. The use of antibiotics of critical importance to human health care (especially cephalosporins of 3rd and 4th generation) is reduced to an absolute minimum. This reduction in usage is reflected in the resistance data of 2016 where resistance levels decreased in *E. coli* from most animal species. Also the occurrence of ESBL/AmpC-producing *E. coli* in poultry meat was substantially lower than in previous years. This suggest that the measure 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. Additional resistance determinants of public health concern such as carbapenemase or the colistine resistance gene *mcr-1*, were not detected or found at low levels, respectively. The ongoing reduction of antibiotic use in livestock in the past seven years is reflected by the ongoing reduction of antibiotic resistance in animals and the food thereof.

2
Usage of antibiotics in animal husbandry in the Netherlands

2.1 Total sales of veterinary antibiotics in the Netherlands 2016

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 on package level sold in 2016 in the Netherlands, as extracted from the Vetindex and supplemented with antimicrobial veterinary medicinal products (AVMP) data of non FIDIN members. The data are estimated to cover approximately 98% of all sales in the Netherlands. Actual use can be different from the quantities sold as a result of stock piling and cross border use. Monitored use in the major livestock farming sectors (pigs, broilers, turkey, veal calves, dairy- and other cattle) covered 97.3% of sales in 2016.
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 antimicrobial veterinary medicinal products, 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 individual animal species. Detailed information about antibiotic usage by animal species in the Netherlands is reported on in the next chapter.

The average number of food-producing animals present in the Dutch livestock farming sector (pigs, poultry, veal calves, other cattle and sheep) shows annual variations (Table ABuse01). In pigs, a decrease is noted in line with international market developments, and in dairy cattle a major increase occurred due to the abandoning of milk quota. This increase will be reversed the coming year because of phosphate production limitations. All in all this indicates that the reported reduction in sales of antimicrobials over the years can be interpreted as true reduction in usage.

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
Piglets (less than 20 kg)	4,809	4,649	4,797	4,993	4,920	5,115	5,408	4,986
Sows	1,100	1,098	1,106	1,081	1,095	1,106	1,053	1,022
Fattening pigs	6,199	6,459	6,200	4,189	4,209	4,087	4,223	4,140
Other pigs	2,100	2,040	2,021	1,841	1,789	1,765	1,769	1,733
Turkeys	1,060	1,036	990	827	841	794	863	762
Broilers	52,323	54,367	57,811	43,912	44,242	47,020	49,107	48,378
Other poultry	46,383	48,218	40,442	52,356	54,345	56,924	58,636	57,172
Veal calves	886	921	906	908	925	921	909	956
Other cattle	3,112	3,039	2,993	3,045	3,064	3,230	3,360	3,353
Dairy cattle	1,562	1,518	1,504	1,541	1,597	1,610	1,717	1,794
Sheep	1,091	1,211	1,113	1,093	1,074	1,070	1,032	1,032
Fattening rabbits	271	260	262	284	270	278	333	318
Dows	41	39	39	43	41	43	48	45

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. Sales of antimicrobial veterinary medicinal products in 2016 (176 tonnes) showed a remarkable reduction (15%) compared to 2015 (206 tonnes). Total sales decreased by 64.45 % over the years 2009-2016. Some of the unexpected increases of 2015 were reversed.
Most classes of antibiotics showed a decrease in 2016, but some increased (Figure ABuse02). Increased sales were noted for 1st and 2nd generation cephalosporins (+12%), amphenicols (+7%), other (+6%) and macrolides (+4%). Reductions in sales were realized for 3rd and 4th generation cephalosporins (-85%), polymyxins (-35%), tetracyclines (-24%), quinolones (-20%), aminoglycosides (-15%), fluoroquinolones (-15%), combinations (-14%), macrolides (-17%), penicillins (-15%) and trimethoprim/sulfonamides (-7%).

Tetracyclines

The total mass of tetracyclines sold decreased considerably more than decrease in use. Since the sales of 2015 increased, in contrast to the decreased use in that year, this likely affected the 2016 figure.
The fraction of doxycycline had increased to 50% of the total sales of tetracyclines (42% in 2015, 41% in 2014, 31% in 2013, 41% in 2012 and 34% in 2011).

Trimethoprim/sulfonamides

The use of trimethoprim/sulfonamides decreased further in 2016, but regained the second rank in mass sold.

Figure ABuse01 Antimicrobial veterinary medicinal product sales 1999-2016 in kg (thousands)

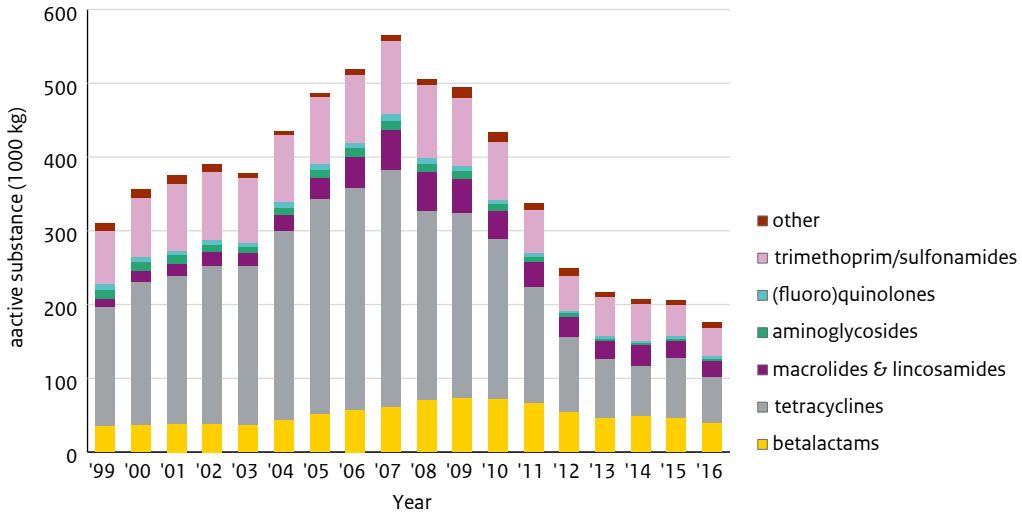
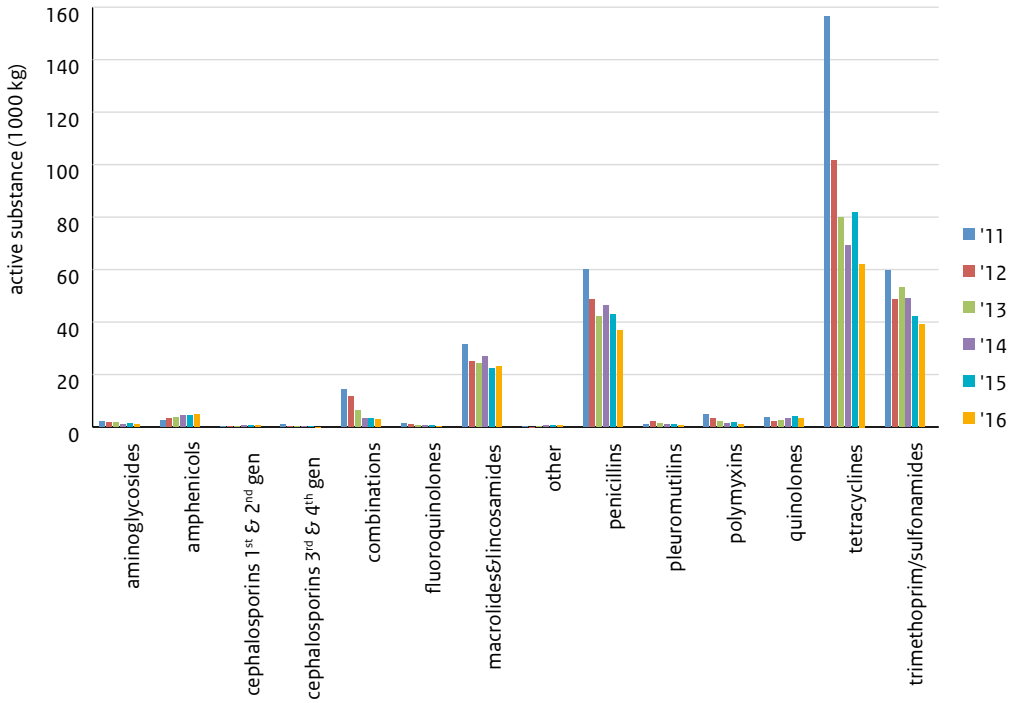


Table ABuse02 Antimicrobial veterinary medicinal product sales from 1999-2016 in kg (thousands) (FIDIN, 2017).

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

Figure ABuse02 Antimicrobial veterinary medicinal product sales by pharmaco-therapeutic class 2011-2016 in kg (thousands)



Penicillins

Now third place in mass again, penicillin sales decreased 20% compared to 2015. 44% of the mass in this group consists of broad spectrum penicillins, compared to 70% previous years. These changes follow the introduction of, and are in line with, the guideline “Dry cow treatment”, endorsing selective use of dry cow treatment and the shift from broad spectrum dry cow treatments to small spectrum dry cow treatments.

(Fluoro)quinolones

The sales of fluoroquinolones decreased with 60 kg in 2016. An overall reduction of 78% was realized in comparison with 2011. 43.2% of the sales are applied in the monitored sectors. The sales of quinolones decreased also, compared with 2011 an overall decrease of 16% 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 presentations of veterinary medicinal product for companion animals were reported for the first time. Sales of these VMPs were stable with a slight decrease in 2015 and an increase in 2016. The sales of 3rd and 4th generation cephalosporins decreased in 2016 with 9 kg, a reduction of 99.8% was achieved since 2011. After this enormous reduction in sales, only 30% (was 83.5%) of 3rd and 4th generation cephalosporin use was not traceable to the monitored food producing animal sectors and companion animals. 12% of the mass sold was used in the monitored sectors.

Polymyxins

Colistin sales and use decreased in 2016, compared to 2011 a reduction of 79% was accomplished. This decrease was promoted by the withdrawal of all oral veterinary medical products of colistin combinations with other antimicrobial drugs.

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

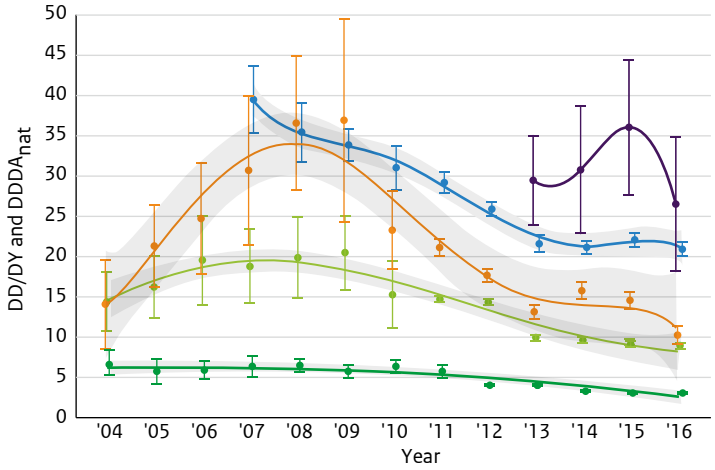
Since 2011, husbandry related consumption reports are prepared by the Netherlands Veterinary Medicines Authority (SDa) using consumption data from *all* farms in the largest sectors of food production animals: pigs, veal calves, broilers and (starting 2012) cattle. Since 2013 also turkeys provided the consumption data, and in 2016 rabbits also joined the monitoring. While the calculation method for treatable 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 veterinary medicinal products consumption data are reported for in 2012 – 2016 (pigs, veal calves, cattle, broilers, turkeys and rabbits). Table ABuse04 depicts the animal bodyweights applied in the calculation of the denominator. In Table ABuse05 the resulting $DDDA_{NAT}$ are shown. In all but one sectors (veal valves, dairy cattle, pigs broilers and turkeys) a reduction in consumption was realized. In other cattle increased consumption is noted, albeit this consumption is low.

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. $DDDA_{NAT}$ 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 2016, 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
pigs	710,688	710,802	704,937	706,025	686,638
veal calves	156,602	159,547	158,828	156,751	164,890
diary cows	924,600	958,200	966,000	1,030,200	1,076,400
other cattle	597,900	573,800	649,000	649,800	600,100
broilers	43,846	44,242	47,020	49,107	48,378
turkeys	4,961	5,046	4,763	5,178	4,572
rabbits	872	830	860	1,004	948

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 2011-2016 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, except for turkey because of broad interval due to small number of farms).



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 ABuse06 depicts the animal bodyweights applied in the calculation of the denominator of $DDDA_F$ by the SDa.

This year the developments in colistin usage over the last four years was reviewed, in view of the international discussion about plasmid bound resistance and the report of ESVAC 2014. In general the usage is low in all sectors, range 0.2% - 3.2% of total use. In pigs the usage was the highest, 0.28 DDDA/year, this corresponds with 0.559 mg/PCU. Thus, colistin usage is below the lowest ESVAC-EMA benchmark for use on the sector level of 1 mg/PCU.

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	

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

	Animalsector														
	Veal calfs*					Dairy cattle					Cattle				
Year	2012	2013	2014	2015	2016	2012	2013	2014	2015	2016	2012	2013	2014	2015	2016
Number of farms with prescriptions	2175	2125	2061	1978	1928	18053	18005	17747	17737	17529	14201	13644	13359	12971	12548
Pharmacotherapeutic group															
Aminoglycosides	0.81	0.53	0.34	0.19	0.23	0.00	0.00	0.00	0.01	0.01	0.03	0.02	0.01	0.01	0.01
Amphenicols	1.23	1.23	1.52	1.63	1.59	0.04	0.05	0.06	0.06	0.06	0.07	0.11	0.10	0.10	0.11
Cefalosporins 1st & 2nd generation	-	-	-	-	-	0.04	0.03	0.02	0.02	0.03	0.00	0.00	0.00	0.00	0.00
Cefalosporins 3rd & 4th generation	0.00	0.00	0.00	-	-	0.04	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00
Combinations	0.42	0.09	0.01	0.00	0.00	1.30	1.01	0.48	0.42	0.38	0.14	0.08	0.04	0.03	0.03
Fluoroquinolones	0.31	0.03	0.02	0.02	0.03	0.01	0.00	0.00	0.00	0.00	0.01				
Macrolides/lincosamides	3.91	3.84	3.72	3.88	3.54	0.07	0.06	0.10	0.10	0.07	0.13	0.22	0.20	0.16	0.17
Other	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Penicillins	2.80	2.11	2.15	2.33	2.25	1.86	2.20	2.00	1.87	1.86	0.22	0.19	0.18	0.16	0.16
Pleuromutilins	-	-	-	-		-	-	-	-	-	-	-	-	-	-
Polymyxins	0.73	0.36	0.15	0.19	0.07	0.06	0.02	0.01	0.01	0.01	0.05	0.01	0.01	0.01	0.00
Quinolones	0.27	0.30	0.49	0.58	0.66	0.00	0.00	0.00	0.00	0.00	0.01	0.01	0.03	0.02	0.03
Tetracyclines	12.61	10.87	10.66	11.01	10.47	0.43	0.42	0.39	0.37	0.35	0.55	0.59	0.47	0.42	0.44
Trimethoprim/sulfonamides	2.76	2.14	2.08	2.22	2.05	0.20	0.22	0.24	0.25	0.24	0.16	0.16	0.11	0.10	0.10
Total	25.85	21.50	21.15	22.05	20.88	4.06	4.03	3.30	3.11	3.01	1.37	1.40	1.15	1.00	1.07

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

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

	Animalsector														
	Pigs					Broilers					Turkeys				Rabbits
Year	2012	2013	2014	2015	2016	2012	2013	2014	2015	2016	2013	2014	2015	2016	2016
Number of farms with prescriptions	6425	6588	6072	5824	5462	732	770	797	816	849	41	40	47	43	43
Pharmacotherapeutic group															
Aminoglycosides	-	-	0.01	0.01		0.58	0.03	0.03	0.02	0.01	1.24	0.40	0.71	0.69	9.66
Amphenicols	0.06	0.09	0.17	0.18	0.24	-	-	-	-	-	0.02	-	-	-	0.00
Cefalosporins 1st & 2nd generation	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Cefalosporins 3rd & 4th generation	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Combinations	0.27	0.10	0.05	0.04	0.03	0.52	0.37	0.08	0.11	0.05	-	-	-	-	-
Fluoroquinolones	0.00	0.00	0.00	-	0.00	0.80	0.24	0.18	0.07	0.07	1.76	1.29	1.20	1.60	0.25
Macrolides/lincosamides	1.39	1.02	1.09	1.04	1.08	1.06	0.31	0.35	0.48	0.25	3.55	2.12	1.98	1.18	1.08
Other	-	-	-	-	-	-	-	-	-	-	-	-	-	-	16.37
Penicillins	2.91	2.17	2.05	1.93	1.97	7.46	6.34	9.96	8.44	6.48	9.34	14.89	16.61	13.75	-
Pleuromutilins	0.35	0.12	0.09	0.08	0.07	-	-	-	-	-	-	-	0.12	-	1.38
Polymyxins	0.58	0.44	0.34	0.38	0.28	0.84	0.08	0.05	0.06	0.04	0.18	0.08	0.63	0.61	0.09
Quinolones	0.03	0.03	0.05	0.03	0.02	1.97	1.65	2.22	2.86	1.51	0.23	0.02	0.10	0.01	-
Tetracyclines	6.79	4.58	4.34	4.15	4.07	2.40	2.52	1.77	1.49	1.01	11.19	9.58	12.57	7.63	10.49
Trimethoprim/sulfonamides	1.92	1.40	1.33	1.20	1.10	1.97	1.46	1.45	1.07	0.78	1.80	2.37	2.01	0.95	1.62
Total	14.32	9.97	9.52	9.05	8.87	17.61	13.01	15.76	14.59	10.19	29.31	30.74	35.94	26.42	40.93

* *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	192
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 in pigs, veal calves, cattle, broilers and turkeys in the Netherlands in number of DDDvet per animal-year

A comparison of the number of DDDA with the internationally established ESVAC DDD_{vet} was conducted for the 2016 data, with the denominator of the $DDDA_{NAT}$.

The use is calculated excluding the locally administered veterinary medicinal products 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, veterinary medicinal products consisting of a combination of active substances result in only one treatment day, while in the ESVAC system the application of such product results in one treatment day for every active substance. This is noticeable in the group trimethoprim/sulfonamides in all sectors, except for turkeys. In turkeys predominantly a product with one sulfonamide is applied, with a much lower authorized dose in the Netherlands than the average dose in Europe. Figure Abuse04 and Table Abuse07 depict the results.

Figure ABuse04 Number of $DDDA_{NAT}$ versus DDD_{vet} per animal-year of systemic veterinary medicinal product only (excluding intramammary and intrauterine applications) in 2016

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

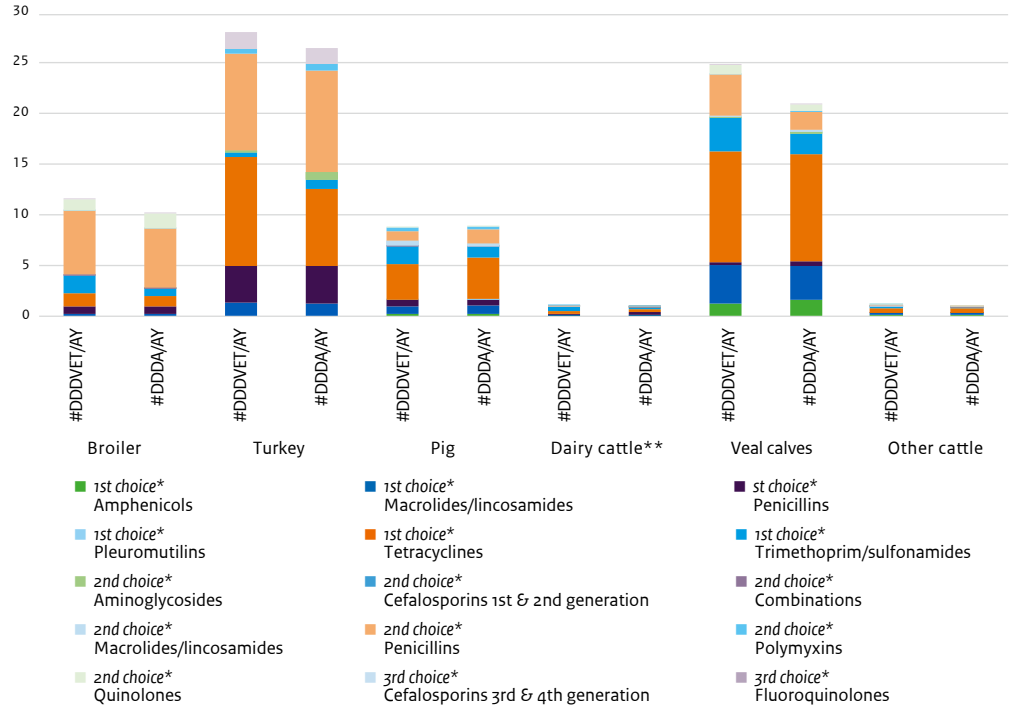


Table ABuse07 comparison number of $DDDA_{VET}$/animal year (AY) and DDDA/AY (=$DDDA_{NAT}$) in monitored sectors.

	Broilers		Turkeys		Pigs		Dairy cattle (excluding intramammary and intrauterine administrations)		Veal calves		Other cattle	
	#DDD_{VET}	#DDDA	#DDD_{VET}	#DDDA	#DDD_{VET}	#DDDA	#DDD_{VET}	#DDDA	#DDD_{VET}	#DDDA	#DDD_{VET}	#DDDA
First choice*	4.02	2.74	16.12	13.46	6.91	6.88	0.95	0.87	19.51	17.94	0.95	0.89
% 1st choice of total	34.84%	26.87%	57.72%	50.95%	79.13%	77.54%	90.33%	90.13%	78.93%	85.90%	81.28%	85.20%
Amphenicols	-	-	-	-	0.18	0.24	0.04	0.06	1.22	1.59	0.09	0.11
Macrolides/lincosamides	0.24	0.25	1.28	1.18	0.81	0.82	0.03	0.05	3.81	3.35	0.17	0.15
Penicillins	0.68	0.70	3.64	3.70	0.57	0.58	0.15	0.26	0.26	0.48	0.05	0.08
Pleuromutilins	-	-	-	-	0.07	0.07	-	-	-	-	-	-
Tetracyclines	1.32	1.01	10.71	7.63	3.46	4.07	0.24	0.27	10.88	10.47	0.47	0.43
Trimethoprim/sulfonamides	1.78	0.78	0.49	0.95	1.81	1.10	0.47	0.24	3.34	2.05	0.17	0.10
Second choice*	7.47	7.38	10.21	11.36	1.82	1.99	0.10	0.09	5.18	2.92	0.22	0.15
% 2nd choice of total	64.55%	72.41%	36.55%	42.99%	20.87%	22.45%	9.34%	9.47%	20.97%	13.97%	18.68%	14.75%
Aminoglycosides	0.00	0.01	0.20	0.69	0.00	0.00	0.01	0.01	0.09	0.23	0.01	0.01
Cefalosporins 1st & 2nd generation	-	-	-	-	-	-	-	-	-	-	-	-
Combinations	1.08	1.51	0.01	0.01	0.02	0.02	0.00	0.00	0.85	0.66	0.04	0.03
Macrolides/lincosamides	0.09	0.05	-	-	0.08	0.03	0.04	0.04	0.00	0.00	0.03	0.03
Penicillins	-	-	-	-	0.41	0.26	0.01	0.01	0.12	0.19	0.01	0.02
Polymyxins	6.28	5.78	9.56	10.05	0.97	1.39	0.04	0.03	4.05	1.77	0.13	0.06
Quinolones	0.03	0.04	0.44	0.61	0.34	0.28	0.01	0.01	0.07	0.07	0.01	0.00
Third choice*	0.07	0.07	1.60	1.60	0.00	0.00	0.00	0.00	0.02	0.03	0.00	0.00
% 3rd choice of total	0.61%	0.72%	5.73%	6.06%	0.00%	0.00%	0.33%	0.40%	0.10%	0.13%	0.03%	0.05%
Cefalosporins 3rd & 4th generation	-	-	-	-	-	-	0.00	0.00	-	-	0.00	0.00
Fluoroquinolones	0.07	0.07	1.60	1.60	0.00	0.00	0.00	0.00	0.02	0.03	0.00	0.00
Total	11.57	10.19	27.93	26.42	8.73	8.87	1.05	0.97	24.72	20.88	1.17	1.04

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

2.4. Usage of antimicrobial veterinary medicinal products in unmonitored sectors.

Surveys were performed in companion animals and horses and the results were published in 2016. Both sectors showed relatively low prescription rates. The consumption of antimicrobials, based on purchased antimicrobial veterinary medicinal products by the veterinary practices in 2014, was for companion animals 2.6 DDDA/animal year, for horses 0.56 DDDA/animal year.

Data from antimicrobial use surveys in sheep and goats of 2012, mink in 2015, zoo-animals in 2016, other poultry (a.o. laying hens) in 2016 and the use of multi-species authorized products in horse and companion animals added up to roughly 10.000 kg mass of active substances. This mass represents the use of veterinary medicinal products apart from the mass used in the monitored sectors and the mass of veterinary medicinal products authorized only for companion animals or horses.

Conclusion

Maximal transparency has been created since 2011 through monitoring antibiotics use by veterinarians and farmers, in 2016 food producing rabbits have joined the regular monitoring as well. The rather steep decrease in sales of antibiotics licenced for therapy in the Netherlands in 2016 may be the result of an adjustment or compensation for the relatively high 2015 sales. The calculation of consumption is based on national conversion factors (DDDA's) of authorized drugs. This year it was possible to allocate most sold antimicrobial veterinary medicinal products to the species the products are used in by using several alternative data sources.

In all but one sectors (veal calves, dairy cattle, pigs broilers and turkeys) a reduction in consumption was realized. In other cattle increased consumption is noted, albeit this consumption is low.

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.

3 Resistance data

This chapter describes susceptibility test results as determined in 2016 for the food-borne pathogens *Salmonella* enterica, *Campylobacter* spp. and *Escherichia coli* O157, and the food-borne commensal organisms *E. coli*, *Enterococcus faecium* and *E. faecalis*. Reduced susceptible and resistant isolates were defined using epidemiological cut-off values (www.eucast.org) 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) should not be automatically classified as clinically resistant. For the purpose of this report we designate all non-wild-type susceptible isolates as "resistant", and specify this per antibiotic if necessary.

3.1 Food-borne pathogens

3.1.1 *Salmonella*

This chapter presents resistance percentages of *Salmonella* isolates, sampled from humans suffering from clinical enteral infections, 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 2016 *S.* Enteritidis (N = 438) followed by *S.* Typhimurium (N = 260) together with the monophasic variant of Typhimurium: *S. enterica subspecies enterica* 1,4,5,12:i:- (N = 229), were most frequently isolated from humans suffering from salmonellosis.
2. In pigs, the monophasic variant of *S.* Typhimurium dominated. In cattle, besides the *S.* Typhimurium variants, *S.* Dublin was most commonly isolated.
3. In poultry (including poultry products, broilers and layers), the number of *S.* Paratyphi B var. Java further reduced in 2016. Also *S.* Infantis, still predominant in 2015, was less frequently isolated in 2016. The prevalence of *S.* Enteritidis increased compared to 2015 and was the most predominant serovar in poultry in 2016.
4. Highest resistance levels were observed in *S.* Kentucky (travel related), the monophasic *S.* Typhimurium, other *S.* Typhimurium, *S.* Paratyphi B var. Java and to a lesser extent in *S.* Infantis and *S.* Newport.
5. Ciprofloxacin resistance was most common amongst isolates from humans and poultry. Predominant serovars were *S.* Enteritidis (23%), *S.* Typhimurium (18%) and *S.* Kentucky (11%).
6. In 2016, the percentage cefotaxime resistant (MIC > 0.5 mg/L) ESBL suspected *Salmonella* isolates was 1.7%, among eleven different serovars, predominantly isolated from human and poultry sources.
7. In 2016 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.

Human isolates tested (N = 1473 in 2016) were a selection of all isolates sent to the RIVM by regional public health and other clinical laboratories. These strains were the first isolates recovered from patients with salmonellosis. The majority of the isolates from pigs (N = 36) and cattle (N = 36) were a random selection sent to the RIVM by the Animal Health Service in Deventer from a diversity of surveillance programs and clinical *Salmonella* infections in animals. Those from poultry (N=122) (and broilers, N = 4; layers, reproduction animals, N = 39) 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 = 385 from animal feed and food products; other animals from animal husbandry (e.g. horses, sheep, goats, ducks) and pets, samples from the environment etc.) have also been serotyped and tested. In addition, NVWA tested 135 *Salmonella* isolates obtained from raw meats, vegetables and seeds. The results of these isolates were not included in Tables S02, S03, S04 and S05, but are depicted in Table S06.

Traditionally, *S.* Enteritidis and *S.* Typhimurium are the most frequently isolated serovars from human clinical infections. This did not change in 2016: *S.* Enteritidis (28.7%) followed by *S.* Typhimurium (17%) together with the monophasic variant of Typhimurium, (*S. enterica subspecies enterica* 1,4,5,12:i:-) (15%), were most frequently isolated from humans suffering from salmonellosis. Referring to *S.* Enteritidis a large European outbreak was caused by consumption of imported eggs from Poland in Dutch

restaurants. Also noticeable is the multi-country outbreak of *S.* Bovismorbificans which could be traced back to a of a raw ham product from Belgium.
S. Typhimurium and its monophasic variant were predominantly associated with pigs and cattle, but was also found in poultry. *S.* Enteritidis was mainly isolated from poultry and layers (Table S01). In pigs, *S.* Typhimurium and its monophasic variant were most predominant. In cattle, besides the *S.* Typhimurium variants, *S.* Dublin was most commonly isolated. In poultry (predominantly layers), *S.* Enteritidis was by far the most prevalent serotype (62.2% of isolates), followed by monophasic variant of Typhimurium (9.6%). The presence of *S.* Paratyphi B var. Java (*S.* Java) and *S.* Infantis was substantially reduced (6.0% and 4.8% respectively).
Depending on the serotype, reported travel contributed up to 39% of the cases of human salmonellosis over the years 2013-2016. Relative high contributions (≥25%) were noted for the serovars Typhi/ Paratyphi A,B (including var. Java),C, Livingstone, Stanley, Tennessee, Weltevreden, Corvallis and Virchow. It should be noted that the contribution of travel as depicted in Table S01 is only indicative of the true contribution, because travel is underreported by an estimated factor of about two.

Resistance levels

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 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 usage of colistin in veterinary medicine has been under discussion and measurements have been taken to reduce the use in animals. Moreover, the recent finding of a plasmid mediated colistin resistance gen (*mcr-1*) resulted in even more attention for this compound. However, like in former years, colistin resistance is not reported in *Salmonella*. That is because a general epidemiological cut-off value is lacking for colistin, the results are 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 for colistin). As a result, colistin resistance would have been over reported in *Salmonella*.

Table S01 Most prevalent *Salmonella* serotypes isolated in 2015 and 2016 from humans, pigs, poultry, broilers (including poultry products) and layers (including reproduction animals and eggs) and the % travel related human infections.

		Travel related	Humans		Pigs		Cattle	
		2013-2016	2015	2016	2015	2016	2015	2016
N Total			1204	1528	54	48	54	45
N tested	Tested		1140	1473	51	36	54	36
Enteritidis	802	13%	283	438				1
Typhimurium	635	5%	233	260	28	10	30	15
Typhimurium (monofasisch)	518	5%	176	229	22	28	7	13
Infantis	181	9%	52	37				
Paratyphi B var. Java	101	20%	19	34				
Dublin	87	4%	21	28		1	15	9
Derby	81	8%	18	20	2	2		
Agona	59	15%	12	13				
Senftenberg	59	24%	3	5				
Typhi/Paratyphi A,B,C	57	28%	22	31				
Brandenburg	54	4%	8	11		2	1	3
Kentucky	53	17%	11	36				
Napoli	52	9%	16	31				
Newport	48	22%	14	23				
Bovismorbificans	39	8%	5	42		1		
Livingstone	37	30%	4	5	1	1		
Stanley	36	25%	21	14				
Saintpaul	34	11%	13	13				
Chester	33	17%	14	16				
Schwarzengrund	33	5%	5	9				
Heidelberg	32	10%	4	5				
Mbandaka	27	17%	2	6				
Tennessee	27	29%		1				
Anatum	26	15%	4	1				
Montevideo	26	24%	5	4				
Goldcoast	25	12%	11	8	1	1	1	2
Rissen	23	11%	10	5		2		
Thompson	22	16%	8	9				
Braenderup	21	19%	9	12				
Oranienburg	21	15%	18	5				
Weltevreden	21	29%	4	10				
Hadar	20	21%	15	5				
Corvallis	19	39%	7	9				
Bredeney	18	25%	5	4				
Give	18	10%	4	4				
Panama	18	14%	7	4				
Muenchen	16	22%	8	2				1
Bareilly	14	7%	5	6				
Virchow	14	29%	6	9				
Indiana	13	9%	4	3				
Kedougou	13	0%	1					
Goettingen	12	0%	5	3				

Table S01 (continued) Most prevalent *Salmonella* serotypes isolated in 2015 and 2016 from humans, pigs, poultry, broilers (including poultry products) and layers (including reproduction animals and eggs) and the % travel related human infections.

	Poultry		Broiler		Layer		Other	
	2015	2016	2015	2016	2015	2016	2015	2016
N Total	1204	1528	54	48	54	45	54	45
N tested	1140	1473	51	36	54	36	54	36
Enteritidis	41	155	8	10	20	84	212	63
Typhimurium	9	8	2		5	2	39	44
Typhimurium (monofasisch)	10	24	9	13		3	20	47
Infantis	32	12	20		3	2	58	95
Paratyphi B var. Java	27	15	14			2	38	25
Dublin	1	1	1				5	14
Derby	9		1		1		20	38
Agona	5		2		2		25	18
Senftenberg	1		1				69	29
Typhi/Paratyphi A,B,C								
Brandenburg	2		2				26	45
Kentucky	1						4	6
Napoli	1						2	3
Newport	1	1					1	4
Bovismorbificans	1		1				1	1
Livingstone	2		1				44	146
Stanley							3	3
Saintpaul	1						3	8
Chester	1		1				1	7
Schwarzengrund	2						5	16
Heidelberg	18		8				14	4
Mbandaka	2	1			2	1	36	17
Tennessee		1		1			34	43
Anatum	1	2	1			1	16	68
Montevideo	1	2				1	10	31
Goldcoast							2	1
Rissen							3	8
Thompson	3				2		1	4
Braenderup	1				1		1	2
Oranienburg							8	7
Weltevreden							10	6
Hadar		1					4	7
Corvallis	1	2	1	1			1	2
Bredeney							3	18
Give		2					5	6
Panama		2				2	5	4
Muenchen	1						2	3
Bareilly							3	
Virchow	2						7	5
Indiana		2				2	10	3
Kedougou	5						1	19
Goettingen	2				1			

Table S01 (continued) Most prevalent *Salmonella* serotypes isolated in 2015 and 2016 from humans, pigs, poultry, broilers (including poultry products) and layers (including reproduction animals and eggs) and the % travel related human infections.

		Travel related	Humans		Pigs		Cattle	
		2013-2016	2015	2016	2015	2016	2015	2016
N Total			1204	1528	54	48	54	45
N tested	Tested		1140	1473	51	36	54	36
Javiana	11	9%	6	5				
Mikawasima	11	0%	7	4				
Kottbus	10	20%	2	5				
Putten	10	n.a.						
Blockley	9	8%	10					
Cerro	9	0%		1				
Poona	9	18%	2	6				
London	8	11%	3	1				
Jerusalem	7	n.a.						
Ohio	6	0%		1				
OVERIGE	278	197%	82	95				1

Table S02 shows MIC-distributions and resistance percentages of 1954 *Salmonella's* from different sources tested for susceptibility in 2016. Highest levels of resistance were observed for sulfamethoxazole, tetracycline, ampicillin, and to a lesser extent for ciprofloxacin, nalidixic acid, chloramphenicol and trimethoprim. The levels of resistance to ciprofloxacin and cefotaxime/ ceftazidime have slightly increased compared to 2015, and seem to fluctuate a little since 2013, but are still higher than in 2012. No resistance to the carbapenem antibiotic meropenem was detected, indicating that carbapenemase producers were not present in the tested isolates (see also chapter 4.2). Similar to 2015 low levels of resistance were found for tigecycline (1.0%) and azithromycin (0.5%) almost exclusively in human isolates.

Table S03 presents resistance percentages for the twelve most prevalent serovars isolated in the Netherlands in 2016. Resistance profiles varied considerably among serovars. High resistance levels (86.9-88.3%) were observed in the monophasic *S.* Typhimurium and in *S.* Kentucky (66.7-91.7%), and to a lesser extent (40.7-45.9%) in *S.* Typhimurium.
Most serovars have acquired resistance against a number of antimicrobials. The most common pattern was resistance to ampicillin, sulfamethoxazole and tetracycline (ASuT). High resistance levels for quinolones (ciprofloxacin and nalidixic acid) were regularly found in *Salmonella*, especially in *S.* Kentucky (travel related), and to a lesser extent in *S.* Infantis, *S.* Newport, *S.* Typhimurium, *S.* Paratyphi B variant Java and *S.* Enteritidis. Highest percentage of fluoroquinolone resistance (18 and 13%) were found amongst human isolates and in poultry (including broilers and layers), reflecting the usage of quinolones in humans and the poultry production chain.

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* spp, based on clinical evidence that there is a poor therapeutic response in systemic infections caused by *Salmonella*

Table S01 (continued) Most prevalent *Salmonella* serotypes isolated in 2015 and 2016 from humans, pigs, poultry, broilers (including poultry products) and layers (including reproduction animals and eggs) and the % travel related human infections.

	Poultry		Broiler		Layer		Other	
	2015	2016	2015	2016	2015	2016	2015	2016
N Total	1204	1528	54	48	54	45	54	45
N tested	1140	1473	51	36	54	36	54	36
Javiana							1	1
Mikawasima							1	1
Kottbus	1		1				2	
Putten	5		1				2	6
Blockley								
Cerro							8	5
Poona							4	1
London	1		1				12	5
Jerusalem	2	4			1	4	3	2
Ohio	6		1				4	5
OVERIGE	10	14	4	1	1	6	144	240

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, 15.1% of *Salmonella* isolates (N =296/1954), demonstrated a resistant phenotype for ciprofloxacin (Table S02). The dominant serovars of ciprofloxacin resistant isolates were S. Enteritidis (23%), S. Typhimurium (18%) and S. Kentucky (11%), mainly from human sources.
In isolates from retail meat (Table S06) the overall ciprofloxacin resistance percentage was 27%. The majority of the isolates was obtained from chicken (N = 33) or turkey meat (N = 7). In chicken meat S. Infantis (N = 19) was most predominant ciprofloxacin resistant serotype followed by S. Paratyphi Java (N = 4). In turkey meat S. Saintpaul (N = 4) was the predominant ciprofloxacin resistant serotype (data not shown).

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). In 2016, the total number of cefotaxime resistant (MIC > 0.5 mg/L) ESBL suspected *Salmonella* isolates was 28/1954 (1.4%), among nine different serovars. Twenty-six isolates were derived from humans: predominantly S. Kentucky (N = 9), S. Typhimurium (N = 4), monophasic S. Typhimurium (N = 3) and S. Infantis (N = 4). The remaining two isolates were derived from poultry sources (S. Paratyphi B variant Java and S. Infantis).
In isolates derived from retail meat (Table S06) the overall cefotaxime resistance was 7.1%. Almost all cefotaxime resistant isolates were obtained from chicken meat (S. Heidelberg (N = 2), S. Paratyphi B variant Java (N = 1) and S. Minnesota (N = 1) or turkey meat (S. Saintpaul (N = 3). One cefotaxime resistant isolate (S. Molade) was obtained from crocodile meat (data not shown).
In addition, at NVWA eight cefotaxime resistant *Salmonella* isolates identified in raw meat from poultry (N=4), turkey (N=3) and crocodile (N=1). Including these isolates the overall cefotaxime resistance in *Salmonella* is 1.7%.

Table S02 MIC distribution (in %) and resistance percentages (R%) for all *Salmonella*'s (N=1954) tested for antibiotic susceptibility during 2016.

Salmonella	MIC (%) distribution mg/L																		R%	95% CI
N = 1954	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							43.7	31.0	1.5		0.1			23.7					23.8	22-25.7
Cefotaxime					97.4	1.1			0.1	1.4									1.4	0.9-2
Ceftazidime						97.1	1.6	0.1	0.5	0.4	0.4								1.2	0.8-1.7
Gentamicin						90.2	7.1	0.7	0.1	0.3	0.8	0.4	0.6						2.0	1.4-2.7
Tetracycline								73.6	1.7	0.2	0.1	1.8	2.4	20.2					24.4	22.5-26.3
Sulfamethoxazole										41.5	24.9	7.0	1.1	0.1	0.1			25.4	25.4	23.5-27.3
Trimethoprim					75.5	17.1	1.0						6.4						6.4	5.4-7.6
Ciprofloxacin	24.9	58.2	1.7	0.7	6.2	5.0	1.3	0.1	0.2	0.7	1.0								15.1	13.6-16.7
Nalidixic acid									80.1	4.6	2.3	1.7		0.5	10.8				13.1	11.6-14.6
Chloramphenicol										89.0	4.1	0.2	0.2	0.8	5.7				6.9	5.8-8
Azitromycin*								0.8	36.5	59.3	3.0	0.3	0.1	0.2					0.5	0.2-0.9
Colistin**							73.2	19.3	7.1	0.4									-	-
Meropenem		92.8	7.2																0.0	0-0
Tigecyclin					48.8	43.2	7.0	1.0	0.1										1.0	0.6-1.5

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 2016 (N tested).

	Enteritidis (467)	Typhimurium (305)	1,4,[5],12:i:- (282)	Infantis (80)	Dublin (40)	Paratyphi B var Java (37)	Kentucky (36)	Derby (35)	Bovismorbificans (32)	Napoli (32)	Brandenburg (29)	Newport (29)
Ampicillin	2.1	45.6	86.9	7.5	0.0	18.9	66.7	2.9	6.3	0.0	0.0	13.8
Cefotaxime	0.0	1.3	1.1	6.3	0.0	5.4	25.0	0.0	3.1	0.0	0.0	0.0
Ceftazidime	0.0	0.0	1.1	6.3	0.0	5.4	25.0	0.0	3.1	0.0	0.0	0.0
Gentamicin	0.0	2.0	2.1	3.8	0.0	0.0	50.0	0.0	3.1	0.0	0.0	0.0
Tetracycline	1.1	40.7	88.3	18.8	0.0	5.4	72.2	5.7	3.1	0.0	3.4	13.8
Sulfamethoxazole	1.1	45.9	87.2	20.0	2.5	21.6	72.2	22.9	3.1	0.0	0.0	13.8
Trimethoprim	0.4	12.5	8.9	15.0	0.0	16.2	8.3	2.9	3.1	0.0	0.0	13.8
Ciprofloxacin	14.3	17.4	8.5	21.3	0.0	16.2	91.7	5.7	3.1	0.0	3.4	20.7
Nalidixic acid	13.9	14.8	6.0	21.3	0.0	13.5	91.7	2.9	0.0	0.0	3.4	13.8
Chloramphenicol	0.0	26.2	8.9	3.8	2.5	2.7	0.0	2.9	3.1	0.0	0.0	13.8
Azithromycin	0.9	0.0	1.1	0.0	0.0	0.0	2.8	0.0	0.0	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	0.4	0.3	1.4	7.5	2.5	0.0	2.8	0.0	0.0	0.0	3.4	0.0

S. Typhimurium

Table S01 shows that *S.* Typhimurium represents 17.0% (260/1528) of all human *Salmonella* isolates as characterized by the RIVM in 2016. This is a bit less than in 2015 (19.4%), but slightly more than 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.

Resistance in *S.* Typhimurium was very high for ampicillin, tetracycline and sulfamethoxazole, for chloramphenicol in human and cattle isolates, and also for trimethoprim in pig isolates (Table S04). About 26% 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) is substantially higher than 2015 (12%) and more similar to the proportion in previous years. Resistance to the clinical important drug cefotaxime was only seen in isolates from humans at a low level (1.5%). Resistance to fluoroquinolones was frequently present in isolates from humans (19.2%) and pigs (12.5%) and (9.0%) in isolates from cattle. These figures indicate a clear increase of fluoroquinolone resistance in *S.* Typhimurium isolates derived from humans and animals compared to 2015. Resistance to tigecycline was absent in isolates derived from humans, pigs and cattle. The only tigecycline resistant isolate (MIC: 2 mg/L) was collected from a sheep carcass.

Table S04 Resistance percentages of *S.* Typhimurium (N tested) isolated from different sources in 2016.

	S. Typhimurium (305)			
	Humans (266)	Cattle (11)	Pigs (8)	Other sources* (20)
Ampicillin	46.2	36.4	62.5	35.0
Cefotaxime	1.5	0.0	0.0	0.0
Ceftazidime	0.0	0.0	0.0	0.0
Gentamicin	1.9	9.1	0.0	0.0
Tetracycline	38.7	72.7	62.5	40.0
Sulfamethoxazole	44.4	72.7	75.0	40.0
Trimethoprim	10.9	0.0	50.0	25.0
Ciprofloxacin	19.2	9.1	12.5	0.0
Nalidixic acid	16.5	9.1	0.0	0.0
Chloramphenicol	27.1	36.4	25.0	10.0
Azithromycin	0.0	0.0	0.0	0.0
Meropenem	0.0	0.0	0.0	0.0
Tigecycline	0.0	0.0	0.0	5.0

** Other sources includes broilers, laying hens, goats and feed products.*

Resistance levels 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 levels for cefotaxime and gentamicin, although being at low level, showed an increasing tendency as from 2011, and fluctuated from 2014 to 2016 (Figure S01).
Resistance levels in *S.* Typhimurium isolates from animal samples (cattle and pigs shown in figure S01) vary considerably over the years. Levels seemed to decrease from 2013, but an increase was seen in 2016. However, these levels 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 raw eggs and, to a lesser extent, of poultry meat products. 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 poultry (broilers and laying hens) and were similar to the most predominant MLVA types in 2013,2014 and 2015. However, in 2016, the most predominant (N=139) *S.* Enteritidis MLVA type (02-09-07-03-02) was part of the outbreak associated with the consumption of Polish eggs. In contrast to 2014 and 2015, resistance to ciprofloxacin and nalidixic acid was found in laying hens, at approximately the same levels as in the human samples (13 to 14%, Table S05). However, the number of samples from laying hens was very low (15), so the reliability of the given percentage of resistance in these strains is low. Sources of human infection with *S.* Enteritidis are considered to be consumption of contaminated eggs and poultry

food products and travel abroad. *S.* Enteritidis prevalence varies over the years, but is traditionally much higher in layers than in broilers.
Compared to many other *Salmonella* serovars, resistance in *S.* Enteritidis is much lower (Table S03). The trends in resistance of *S.* Enteritidis over the years are summarized in Figure S02. Resistance levels in human isolates showed a small decrease for all antimicrobials, compared to 2015. As seen for *S.* Typhimurium, resistance levels for isolates from laying hens and other sources seem to vary considerably over the years due to the relatively small number of samples per year. It should be realized that these resistance percentages are not very reliable.

Figure S01 Trends in resistance (%) of *S.* Typhimurium isolated from humans and food-animals in 1999-2016.

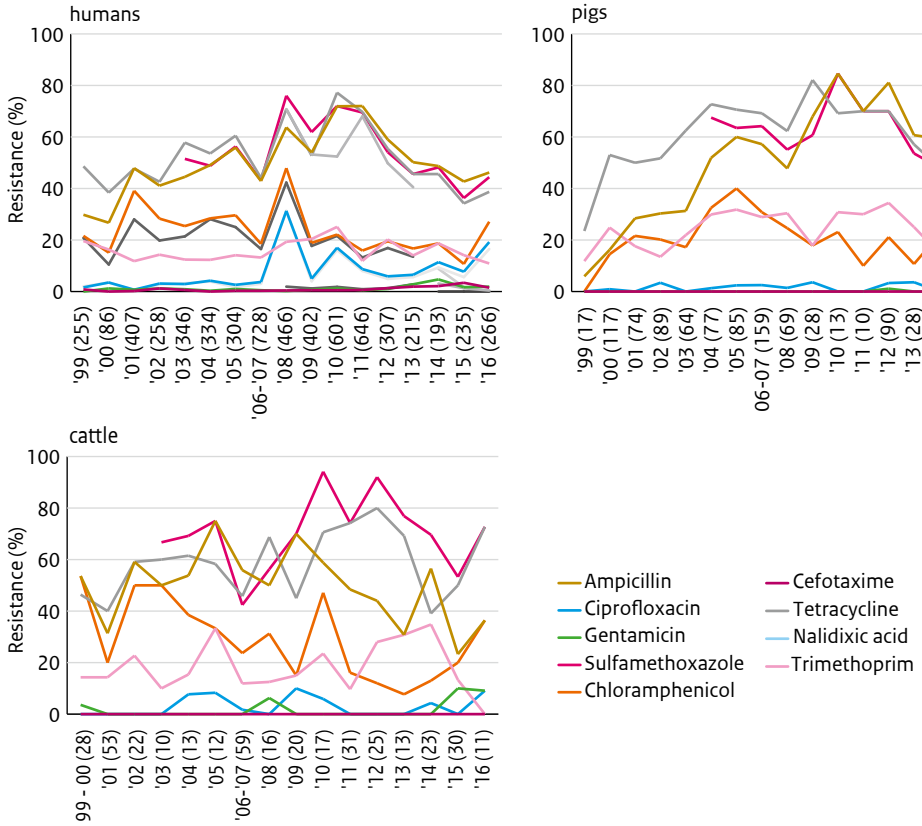


Table S05 Resistance percentages of *S.* Enteritidis (N tested) isolated from different sources in 2016.

	S. Enteritidis (467)		
	Humans (392)	Laying hens (15)	Other sources* (60)
Ampicillin	2.1	0.0	0.0
Cefotaxime	0.0	0.0	0.0
Ceftazidime	0.0	0.0	0.0
Gentamicin	0.0	0.0	0.0
Tetracycline	1.1	0.0	0.0
Sulfamethoxazole	1.1	0.0	0.0
Trimethoprim	0.4	0.0	0.0
Ciprofloxacin	14.3	13.3	13.3
Nalidixic acid	13.9	13.3	13.3
Chloramphenicol	0.0	0.0	0.0
Azithromycin	0.9	0.0	0.0
Meropenem	0.0	0.0	0.0
Tigecycline	0.4	0.0	0.0

** Other sources includes mainly broilers (n = 55), but also isolates from cattle, feed and food products.*

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

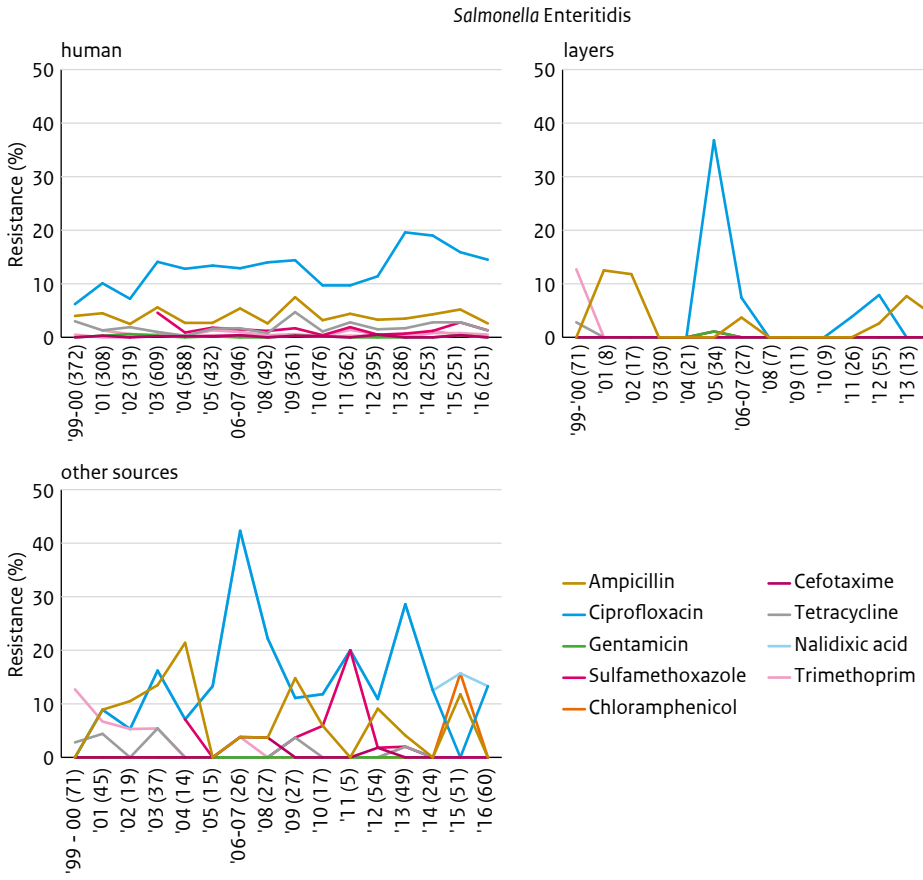
The prevalence of *S.* Java further decreased in 2016. As a consequence, *S.* Java was not the most predominant serovar isolated in broiler production anymore, as it was in the period before 2015. Sixteen *S.* Java strains from poultry were included for susceptibility testing (Figure S03). Resistance levels of most antimicrobials increased, compared to 2015. Since 2012, the resistance levels seem to fluctuate, and a real increasing or decreasing trend cannot be seen. The resistance percentage to trimethoprim was at 100%, like in former years. NB: due to an error in MARAN 2016 resistance percentages of animal isolates (especially for trimethoprim) were wrongly depicted in Figure S03. Resistance against chloramphenicol was not detected in the samples from 2016. The resistance level for the quinolones ciprofloxacin and nalidixic acid were for both 50.0%.
In 2016, 34 *S.* Java strains were isolated from human infections. All strains tested were trimethoprim susceptible and therefore not considered to be related to the clone spreading in Dutch poultry and probably travel related.

Salmonella from chicken meat, pork, other meat sources and herbs and seeds

Resistance data of *Salmonella* isolates from raw meat, herbs and seeds are presented (Table S06, Figure S03). In 2016 *S.* Infantis (40%) was the dominant serovar found in samples from chicken meat, followed by *S.* Enteritidis (18%) *S.* Paratyphi B variation Java (16%). In general, isolates from pork were resistant against a fewer number of antimicrobials than isolates from chicken meat and other raw meat sources (Java excepted). Resistance levels to the quinolones (ciprofloxacin and nalidixic acid) in chicken meat isolates were very high (61.8% and 60.0% respectively); levels in meat from other species were reduced, compared to 2015. Resistance levels to tigecycline were lower than in 2015, and not detected in isolates from herbs and seeds. Resistance to cephalosporins (cefotaxime and ceftazidime) halved in

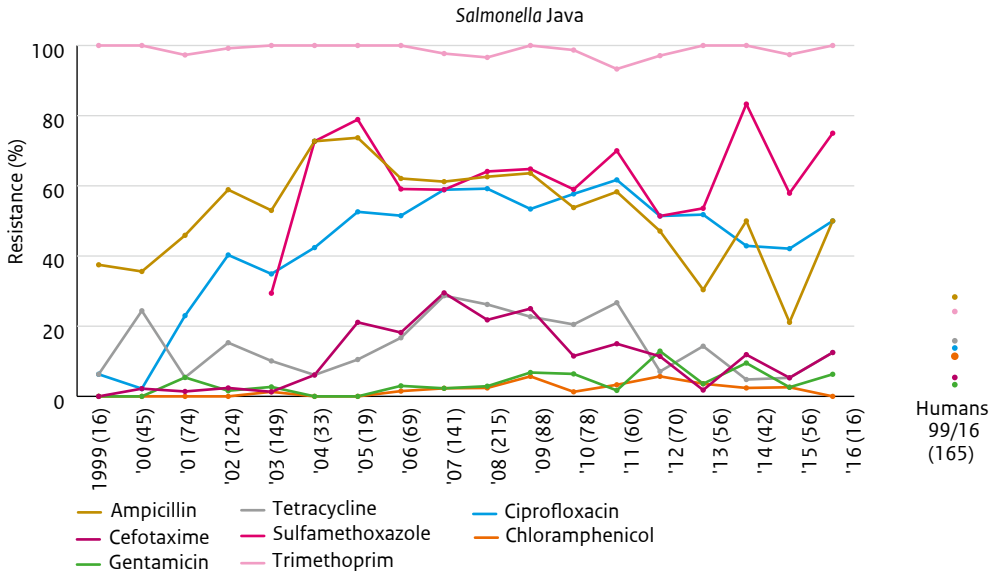
chicken meat and other raw meat since 2015 (7.3% for both in chicken meat and 9.8% for both in other raw meat products), and was absent in pork meat.
Resistance levels to quinolones (ciprofloxacin and nalidixic acid) in herbs and seeds were lower than in 2015 (8.7%); resistance to cephalosporins (cefotaxime and ceftazidime) was not detected in these samples. Eighteen different *Salmonella* serotypes were found among 23 samples from herbs and seeds. Among those were three of the twelve most prevalent serotypes described earlier in Table S03:
S. Enteritidis (n=1), S. Kentucky (n=1) and S. Typhimurium (n=1).
All percentages in TableS06 should be interpreted with care, because of the relatively low number of samples.

Figure S02 Trends in resistance (%) of S. Enteritidis isolated from humans, layers and other sources from 1999-2016.



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

Figure S03 Trends in resistance (%) of S. Paratyphi B var. Java isolated from poultry sources from 1999-2016 and humans (Separate data on the rigt indicate all human S. java isolates from 1999-2016).

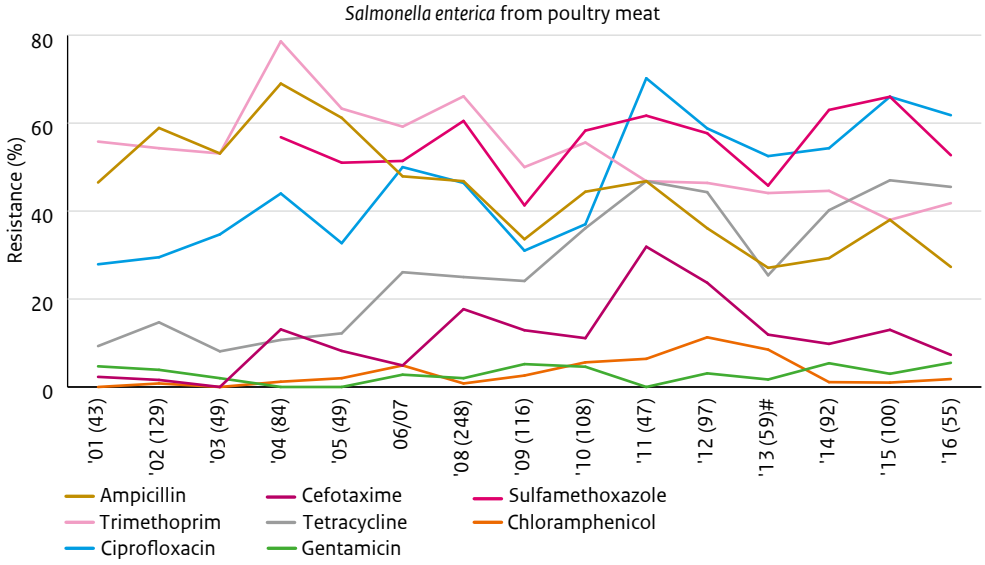


Due to an error in MARAN 2016 resistance percentages of animal isolates (especially for trimethoprim) were wrongly depicted.

Table S06 Resistance (%) of *Salmonella enterica* isolated from different types of raw meat, herbs and seeds in the Netherlands in 2016.

	Chicken N = 55	Pork N = 16	Other meat N = 41	Herbs and seeds N = 23
Ampicillin	27.3	31.3	19.5	0.0
Cefotaxime	7.3	0.0	9.8	0.0
Ceftazidime	7.3	0.0	9.8	0.0
Gentamicin	5.5	0.0	0.0	0.0
Tetracycline	45.5	31.3	19.5	8.7
Sulfamethoxazole	52.7	31.3	22.0	13.0
Trimethoprim	41.8	6.3	14.6	0.0
Ciprofloxacin	61.8	0.0	19.5	8.7
Nalidixic acid	60.0	0.0	19.5	8.7
Chloramphenicol	1.8	0.0	7.3	0.0
Azithromycin	0.0	0.0	2.4	0.0
Meropenem	0.0	0.0	0.0	0.0
Tigecycline	1.8	0.0	4.9	0.0

Figure S04 Trends in resistance (%) of *Salmonella enterica* isolated from poultry meat in the Netherlands from 2001-2016.



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

3.1.2 Campylobacter

This chapter describes the 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 focus of the surveillance of antimicrobial resistance in *Campylobacter* is mainly at poultry (and poultry meat products). In addition to broilers, laying hens and ducks were included in the surveillance of 2016. In 2016 also *C. jejuni* isolates from milk goats and milk sheep were tested for resistance.

Table C01 presents the MIC-distributions and resistance percentages for all *Campylobacter jejuni* and *C. coli* strains isolated at WBVR from caecal samples of broilers, laying hens and ducks in 2016. Resistance percentages of *C. jejuni* and *C. coli* isolated from different faecal and meat sources are shown in Table C02. Trends in resistance of *C. jejuni* and *C. coli* from broilers and broiler 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 2006 onwards in Table C03.

Highlights

1. As a result of prioritization and changes in legislation, since 2014 the focus of the surveillance of antimicrobial resistance in *Campylobacter* is mainly in isolates from poultry (including broilers, laying hens and ducks) and poultry meat.
2. Resistance rates in *C. jejuni* from broilers was somewhat lower, whereas rates in poultry meat did not substantially change in 2016, compared to 2015.
3. Overall, resistance levels were higher in *C. coli* than in *C. jejuni* isolates.
4. Resistance rates for quinolones in *C. coli* isolates from broilers, laying hens and poultry meat decreased since 2015.
5. Levels of resistance of *C. jejuni* for tetracycline and the quinolones were substantially higher in broilers than in ducks and laying hens.
6. In *C. jejuni* from milk sheep and milk goats, resistance percentages were highest for ciprofloxacin, nalidixic acid and tetracycline, but at much lower levels than in poultry.
7. Ciprofloxacin resistance in *Campylobacter* isolates from human patients is still high (with a slight decrease in 2016), 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 levels were higher for all three antimicrobials tested in travel related infections compared to domestically acquired campylobacteriosis.

Resistance levels

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. The remaining six antimicrobials ciprofloxacin and nalidixic acid (quinolones), gentamicin and streptomycin (aminoglycosides), erythromycin (macrolides) and tetracycline (tetracyclines), represent antimicrobial classes, which are all important in human medicine for treatment of campylobacteriosis.

In 2016, the highest resistance levels of *C. jejuni* and *C. coli* in broilers, laying hens and ducks were detected for tetracycline and the quinolones ciprofloxacin and nalidixic acid (Table CO1). Table Co2 shows that resistance percentages for the antimicrobials were at high levels for broilers, ducks, poultry and turkey meat (both *C. jejuni* and *C. coli*), a bit lower for isolates from laying hens (only for C. *jejuni*) , and at the lowest levels (around 10%) for *C. jejuni* isolates from faecal samples of milk goats and milk sheep. Resistance in *C. jejuni* from broilers and poultry meat seems to have stabilized to very low levels for erythromycin, streptomycin and gentamicin: resistance to erythromycin and gentamicin could not be detected in broilers, as was the case for gentamicin and streptomycin in poultry meat. Resistance to tetracycline showed a slight decrease since 2013, although in 2016 the resistance percentage in poultry meat was a bit higher than in 2015. However, the resistance percentage of *C. jejuni* to tetracycline was still high (46.5% in broilers and 42.3% in poultry meat). Resistance to ciprofloxacin showed more fluctuation over the years and was over 60% since 2014 (Figure Co1).

More fluctuation over the years was observed in *C. coli* from broilers and poultry meat than in C. *jejuni*, probably due to the relatively low number of isolates in the survey (Figure Co2). However, resistance in *C. coli* from broilers stabilized to low levels for erythromycin, streptomycin and gentamicin, and was not detected in poultry meat samples in 2016. Resistance percentages for ciprofloxacin in broilers have been fluctuating a lot since 2001, with 65.1% resistant isolates in 2016. Resistance percentages for ciprofloxacin in poultry meat showed a substantial decrease in 2016 to 56.5%, after having been between 78% and 83% since 2010. However, because of the low number of *C. coli* isolates tested in 2016 (N = 23) these results should be interpreted with care. Resistance levels to tetracycline in broilers and poultry meat seem to follow the same trend as ciprofloxacin resistance, at approximately equal percentages (Figure Co2).

Overall, resistance levels were higher in *C. coli* than in *C. jejuni* isolates (Table Co1 and Co2). Table Co2 shows that resistance against gentamicin was not detected in any of the *C. coli* isolates and in the *C. jejuni* isolates only in 4.8% of the turkey meat samples. Resistance against streptomycin and erythromycin was also at low levels, except for the streptomycin resistance percentage in *C. jejuni* isolates from turkey meat (14.3%) and the *C. coli* isolates from laying hens and ducks (6.9% and 6.3% respectively).

Table C01 MIC distribution (in %) for *Campylobacter jejuni* (N = 309) and *C. coli* (N = 146) isolated from caecal samples of broilers, layers and ducks in 2016 .

C. *jejuni*	MIC (%) distribution mg/L												R%	95% CI
(N = 309)	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256		
Ciprofloxacin	42.1	5.5	1.0	0.0	0.0	0.3	17.8	19.7	13.6				51.5	45.7 - 57.1
Nalidixic acid				0.0	10.0	36.6	4.5	0.0	0.0	0.0	48.9		48.9	43.1 - 54.5
Erythromycin				86.4	12.6	1.0	0.0	0.0	0.0	0.0	0.0		0.0	0 - 0
Gentamicin	76.4	23.6	0.0	0.0	0.0	0.0	0.0	0.0					0.0	0 - 0
Streptomycin		10.0	61.5	27.5	0.0	0.0	0.3	0.3	0.3				1.0	0 - 2
Tetracycline			58.6	2.6	0.0	0.0	0.0	1.0	3.6	4.9	29.4		38.8	33.2 - 44.3

C. *coli*	MIC (%) distribution mg/L												R%	95% CI
(N = 146)	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256		
Ciprofloxacin	34.2	5.5	0.0	0.0	0.0	8.9	36.3	13.7	1.4				60.3	52.1 - 68.3
Nalidixic acid				0.0	3.4	25.3	11.0	0.0	0.0	6.2	54.1		60.3	52.1 - 68.3
Erythromycin				76.0	19.2	1.4	0.0	0.0	0.0	1.4	0.0		3.4	0.4 - 6.4
Gentamicin	18.5	77.4	4.1	0.0	0.0	0.0	0.0	0.0					0.0	0 - 0
Streptomycin		0.0	13.7	78.8	2.1	0.0	0.7	3.4	1.4				5.5	1.7 - 9.2
Tetracycline			37.0	7.5	0.0	0.0	0.0	0.0	0.7	2.7	52.1		55.5	47.2 - 63.7

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 EUCAST clinical breakpoints.

For tetracycline (only C. coli), ciprofloxacin and erythromycin the ECOFF and clinical breakpoint are identical.

Table C02 Resistance percentages of C. *jejuni* and C. *coli* isolated from faecal samples of broilers, layers, ducks, milk goats and sheep and from meat samples of poultry and turkey in 2016

	C. jenuni								*C. coli*			
	Broilers	Layers	Ducks	Poultry meat	Turkey meat	Milk	goats	Milk sheep	Broilers	Layers	Ducks	Poultry meat
N	170	71	68	52	21	382	43	17	43	87	16	23
Ciprofloxacin	60.6	32.4	48.5	65.4	38.1		9.3	11.8	65.1	56.3	68.8	56.5
Nalidixic acid	59.4	29.6	42.6	65.4	38.1		9.3	11.8	65.1	56.3	68.8	56.5
Erythromycin	0.0	0.0	0.0	3.8	0.0		0.0	5.9	4.7	3.4	0.0	0.0
Gentamicin	0.0	0.0	0.0	0.0	4.8		0.0	0.0	0.0	0.0	0.0	0.0
Streptomycin	1.2	1.4	0.0	0.0	14.3		2.3	0.0	2.3	6.9	6.3	0.0
Tetracycline	46.5	25.4	33.8	42.3	38.1		9.3	5.9	65.1	52.9	43.8	52.2

Quinolones

The increasing trend in resistance to the quinolones of *Campylobacter* spp. isolates from animal origin (Figures Co1 and Co2) as well as from human patients (Figure Co3) is a public health concern. After a period of decreasing ciprofloxacin resistance in *C. jejuni* isolates from broilers (52.2% in 2013), resistance increased to 64.3% in 2014 and 69.6% in 2015. In 2016 a slight decrease to 60.6% was seen. The resistance level of *C. jejuni* from poultry meat is comparably high and also showed an increase to 63.4% in 2014 and 66.0% in 2015, and stabilized at 65.4% in 2016. Ciprofloxacin resistance rates in *C. jejuni* isolates from laying hens were relatively high, but showed a slight decrease from 36.4% in 2015 to 32.4% in 2016. The resistance levels for ducks, goats, sheep and turkey meat cannot be compared to former years, because these samples were collected in 2016 for the first time.

High levels of ciprofloxacin resistance were also observed in *C. coli* isolates from broilers with 51.3% in 2014 and 71.4% in 2015, but like the *C. jejuni* isolates a slight decrease in 2016 to 65.1%. The quinolone resistance for *C. coli* isolates from poultry meat showed a substantial decrease from 78.0% in 2015 to 56.5% for ciprofloxacin and from 84.0% in 2015 to 56.5% in 2016 for nalidixic acid. Also ciprofloxacin resistance in laying hens decreased from 69.3% in 2015 to 56.3% in 2016. The resistance levels for fluoroquinolone in human campylobacter isolates were also high (58.4%), but were also decreased compared to 2014 (60.7%) and 2015 (61.4%).

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

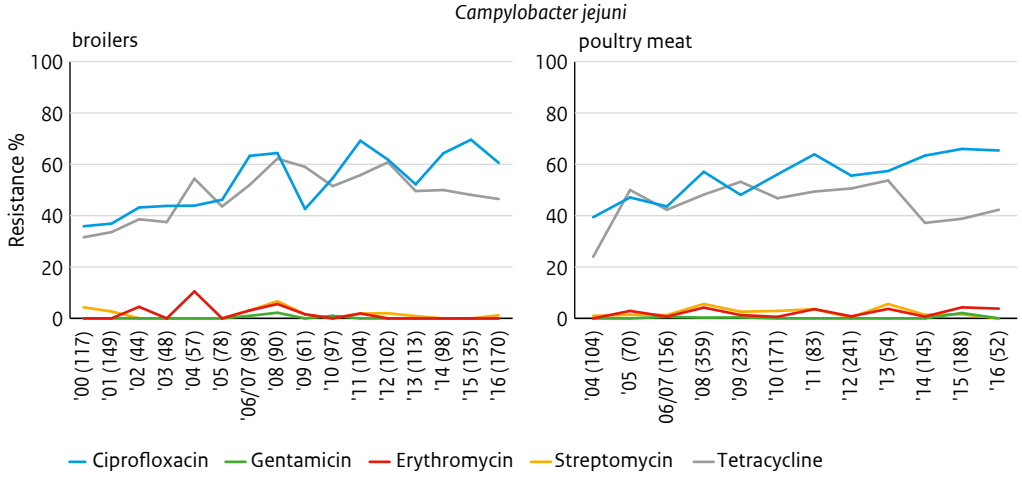


Figure C02 Trends in resistance (%) of *Campylobacter coli* isolated from broilers and poultry meat in the Netherlands.

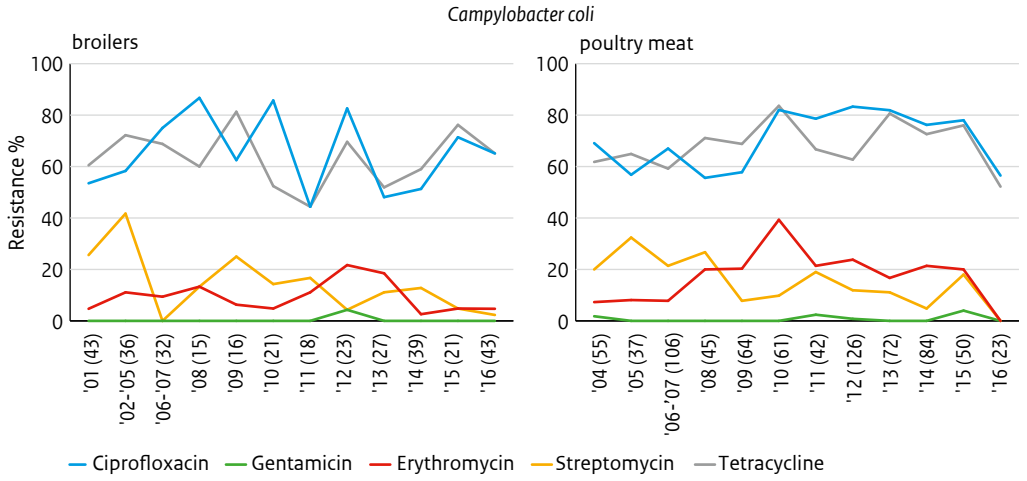


Table C03 Domestically acquired and travel related resistance in C. *jejuni* and C. *coli* isolated from humans from 2006 - 2016 from all 16 Public Health Services (PHLS) covering >50% of the Dutch population.

	2006-2011							
	Domestically acquired				Travel related			
	C. jejuni		*C. coli*		*C. jejuni*		*C. coli*	
	N	R%	N	R%	N	R%	N	R%
Fluoroquinolone	15261	49.9	1127	48.8	786	65.0	83	59.0
Tetracycline	9612	20.2	795	30.6	257	30.0	42	23.8
Erythromycin	12606	2.2	968	6.7	596	4.0	66	12.1

	2012-2016							
	Domestically acquired				Travel related			
	C. jejuni		*C. coli*		*C. jejuni*		*C. coli*	
	N	R%	N	R%	N	R%	N	R%
Fluoroquinolone	14179	58.4	986	62.9	846	73.8	106	70.8
Tetracycline	8310	39.2	569	57.1	393	59.0	52	61.5
Erythromycin	12286	2.0	800	14.0	736	3.8	95	25.3

	Campylobacter spp. (R%)					
	2016	2015	2014	2013	2012	2006/11
Fluoroquinolone	58.4	61.4	60.7	57.6	59.4	49.3
Tetracycline	42.3	42.2	44.3	38.5	35.4	21.2
Erythromycin	2.7	2.9	3.4	3.2	3.0	2.6

Macrolides

Erythromycin, or other macrolides (clarithromycin), are the first-choice drugs for the treatment of campylobacteriosis in humans. The level of resistance to macrolides reported in animals and humans is low for C. *jejuni*, on average 1.3% of strains from broilers, layers, turkey, poultry meat and turkey meat in 2016 and 2.0% of human isolates from 2012-2016 were classified resistant. It should be noted that for human isolates more sensitive 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*).

In 2016, like in former years, erythromycin resistance was low in C. *jejuni* isolates, with no resistance in broilers, laying hens, ducks, milk goats and turkey meat, and 3.8% in poultry meat and 5.9% in milk sheep (Table Co2). Erythromycin resistance in C. *coli* was also low in broilers (4.7%) and laying hens (3.4%), and could not be detected in ducks and poultry meat, which is remarkable, because the resistance percentage of poultry meat isolates in 2015 was 20.0%. Again, this difference could be the effect of the inclusion of imported meat products.

Broiler chickens, laying hens, ducks, poultry meat and turkey meat

In *Campylobacter* spp from poultry, resistance profiles were determined for isolates recovered from animals (broilers, laying hens, ducks) as well as from chicken and turkey meat samples. In laying hens, the antibiotic use is on average considerably less than in broilers.

As shown in Table Co2, levels of resistance of C. *jejuni* for tetracycline and the quinolones were substantially higher in broilers than in laying hens. Resistance levels of C. *jejuni* isolates from ducks for these antimicrobials was lower than in broilers, but higher than in laying hens. However, resistance rates for the quinolones of C. *coli* isolates from broilers, laying hens and ducks were comparable, and reasonably high. The resistance rate for tetracycline in C. *coli* isolates was highest for broilers (65.1%), and somewhat lower for laying hens (52.9%) and ducks (43.8%).

Resistance rates for tetracycline and the quinolones in C. *jejuni* isolates from poultry meat were at the same level as for the isolates from broilers. The resistance percentages for the C. *coli* isolates from broilers were a little higher than for the isolates from meat. Resistance rates for C. *jejuni* isolates for erythromycin, gentamicin and streptomycin were at low levels, except for streptomycin resistance in turkey meat isolates (14.3%). Resistance in C. *coli* isolates streptomycin was 6.9% in laying hens and 6.3% in ducks.

In general, higher resistance rates were observed for most antimicrobials in C. *coli* from broilers, laying hens and ducks compared to C. *jejuni* from the same animals. The difference in resistance of *Campylobacter* spp. isolates from animals and meat products may be due to the inclusion of foreign poultry products in the survey.

Milk sheep and milk goats

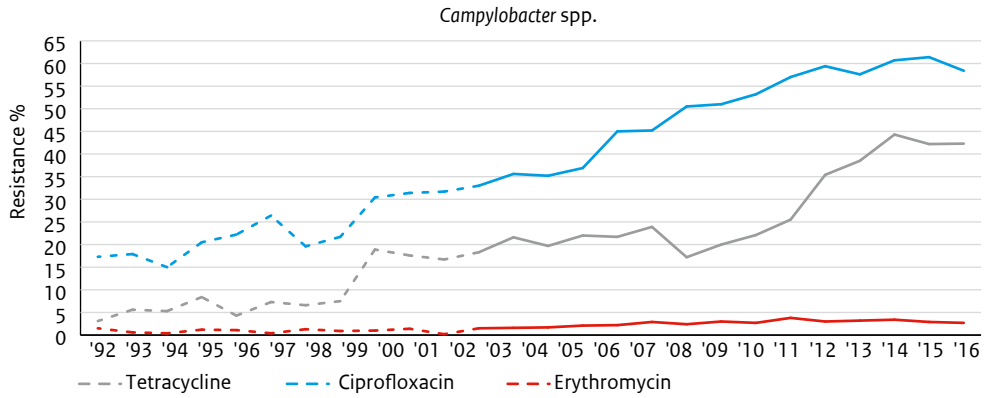
In 2016 for the first time C. *jejuni* isolates from milk goats and milk sheep were tested for antimicrobial resistance. Like in the other animal species, resistance percentages were highest for ciprofloxacin, nalidixic acid and tetracycline, but at much lower levels (9.3% for both quinolones in goats and 11.8% for both quinolones in sheep, for tetracycline 9.3% in goats and 5.9% in sheep) (Table Co2). Although no information is available on the usage of antimicrobials in goats and sheep the low resistance rates probably reflect relatively low use in these animals.

Campylobacter in humans

Data on resistance levels are available for ciprofloxacin, erythromycin and tetracycline and are summarized in Table C03 and Figure C03. The trends as shown in Figure C03 indicate a continuously increasing trend of ciprofloxacin and tetracycline resistance in *Campylobacter* spp. isolated from human patients, with a slight decrease for tetracycline since 2015 and for ciprofloxacin since 2016. 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. After a tendency of increasing resistance to ampicillin, tetracycline, sulfamethoxazole and trimethoprim since 2009 in STEC O157 isolates from humans, in 2016, a decrease was found for ampicillin (from 14.3% to 10.7%), sulfamethoxazole (from 15,6% to 14.7%) and trimethoprim (from 14.3% to 8.0%).
2. Resistance for the quinolones (ciprofloxacin and nalidixic acid) was not detected in human STEC O157 isolates.

Shiga-toxin producing *E. coli* O157 (STEC O157) isolates from humans were tested for susceptibility. MIC results for all *E. coli* O157 isolates from humans are presented in Table STEC01 and the trends over time in Figure STEC01. In 2016, no *E. coli* non-O157 isolates were tested from animals or beef products.

Human STEC O157 isolates

Resistance rates of human isolates showed a tendency to increase for ampicillin, tetracycline, sulfamethoxazole and trimethoprim since approximately 2009 (Figure STEC01). In 2016, a decrease was found for ampicillin (from 14.3% to 10.7%), sulfamethoxazole (from 15,6% to 14.7%) and trimethoprim (from 14.3% to 8.0%). After finding low resistance levels for quinolones in 2013 (4.2%) and 2014 (2.4%), resistance for ciprofloxacin and nalidixic acid was not detected in 2015 and 2016. As in former six years, no ESBL-producing isolates were detected.

Figure STEC01 Trends in resistance (in %) of *E. coli* STEC O157 isolated from humans in the Netherlands from 1999-2016.

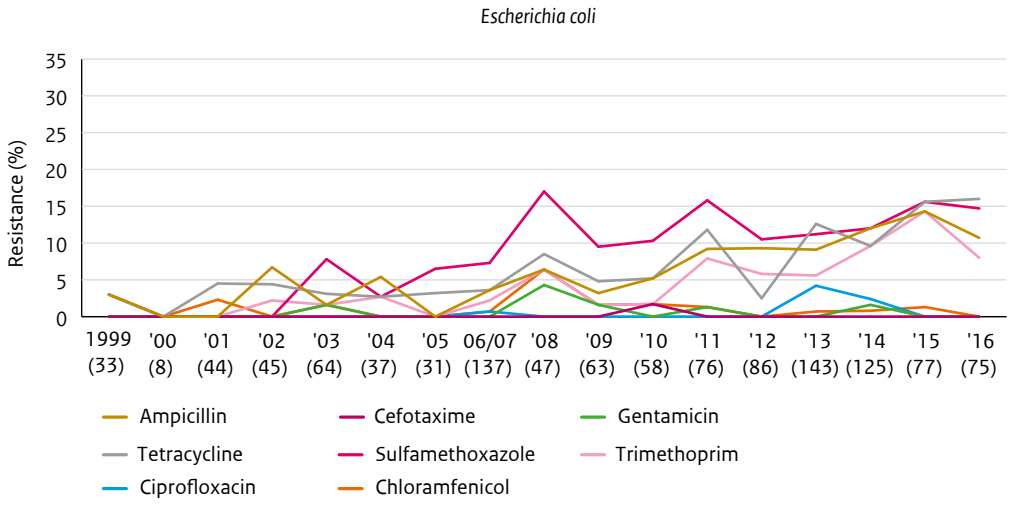


Table STEC01 MIC distribution (in %) and resistance percentages (R%) for *E. coli* STEC O157 (N=75) isolated from humans the Netherlands in 2016.

E. coli	MIC (%) distribution mg/L																	R%	95% CI
N = 77	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								2.7	86.7					10.7					10.7	3.5 - 17.7
Cefotaxime					100														0.0	0 - 0
Ceftazidime						100													0.0	0 - 0
Gentamicin						81.3	16.0	2.7											0.0	0 - 0
Tetracycline								41.3	42.7				1.3	14.7					16.0	7.5 - 24.4
Sulfamethoxazole										85.3								14.7	14.7	6.4 - 22.8
Trimethoprim					92.0								8.0						8.0	1.7 - 14.2
Ciprofloxacin	72.0	28.0																	0.0	0 - 0
Nalidixic acid									100.0										0.0	0 - 0
Chloramphenicol										93.3	6.7				0.0				0.0	0 - 0
Azithromycin*								6.7	70.7	22.7									0.0	0 - 0
Colistin							100.0	0.0											0.0	0 - 0
Meropenem		100																	0.0	0 - 0
Tigecycline					100														0.0	0 - 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, 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 thereof. The level of antimicrobial resistance in bacteria inhabiting the intestinal tract directly reflects the selection pressure as a result of the use of antibiotics in animals, especially over time. For this purpose, *E. coli* and *Enterococcus* species (*E. faecium* and *E. faecalis*) are included as indicator organisms for the Gram-negative and the Gram-positive flora, respectively. As a result of less priority for including enterococci in the surveillance, no enterococci from faecal samples were tested in 2016, but *Enterococcus faecalis* and *E. faecium* were isolated from chicken and turkey meat samples.

Isolation of bacteria from the intestine of randomly picked food-producing animals at slaughter aims to detect the development of resistance at the bacterial population level in food animals as prescribed by EFSA [1]. Since 1998 this monitoring is conducted in slaughter pigs and broilers. From 2005 onwards, resistance in isolates from both dairy cattle, veal calves and meat samples have been included. In the years 2010 and 2011 samples of individual dairy cattle were taken 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 of veal calves were collected at slaughterhouses and resistance levels were reported separately for white veal calves 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 taken 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 animal in the Netherlands.
The EU legislation on monitoring and reporting of antimicrobial resistance in zoonotic and commensal bacteria (2013/652/EU) was implemented in 2014 and 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 2016

1. In 2016, resistance levels of indicator *E. coli* from faecal samples showed a tendency to decrease in broilers and pigs and stabilized in veal calves and dairy cattle.
2. In isolates from chicken meat resistance levels were substantially lower than in isolates from turkey meat. The levels of resistance were similar to 2015 in both types of poultry meat.
3. Resistance levels for almost all tested antibiotics were higher in samples of imported chicken and turkey meat than in samples from retail.
4. Resistance to third-generation cephalosporins was low (< 1%) in all tested animal species.
5. Although resistance to fluoroquinolones is decreasing, it was still commonly present in indicator *E. coli* from broilers and to a lesser extent white veal calves, but substantially decreased, in *E. coli* from white veal calves.
6. Among indicator *E. coli* from animals and meat, resistance levels to ampicillin, tetracycline, sulphonamides and trimethoprim were still reasonably high in broilers, turkey, pigs and veal calves.
7. Levels of resistance in *E. coli* from rosé veal calves were substantially lower than those from white veal calves for almost all antibiotics tested.

Resistance levels

Resistance levels of a total of 1492 *E. coli* isolates obtained from broilers, pigs, dairy cows, veal calves, laying hens and ducks are presented as MIC-distributions in Table Eco01 and as resistance percentages per animal species in Table Eco02. Trends in resistance levels from 1998 to 2016 are shown in Figure Eco01 and information on trends in multidrug resistance is shown in Figure Eco02.
Resistance percentages of 321 *E. coli* isolates collected from raw chicken and turkey meat products are presented in Table Eco03. Trends in resistance of *E. coli* in the Netherlands from 2002 to 2016 isolated from raw meat products of poultry and turkey are presented in Figure Eco03.

For most drugs or drug classes there were notable variations in resistance levels between the different animal species (Table Eco02). Highest levels were present in broilers, slaughter pigs and white veal calves, lower levels for rosé veal calves, laying hens and ducks, and hardly any resistance was observed in isolates from dairy cattle. In general, the highest resistance levels were seen for ampicillin, tetracycline, sulfamethoxazole and trimethoprim. These include the most frequently used drug classes in veterinary medicine in The Netherlands.

Table Eco01 MIC distribution (in %) and resistance percentages (R%) for all *E. coli* (N=1492) isolated as indicator organism from intestines of food producing animals in the Netherlands in 2016.

E. coli	MIC (%) distribution mg/L																	R%	95% CI
N = 1492	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	30.1	45.6	2.5		0.1		20.0					20.2	18 - 22.2
Cefotaxime					99.7	0.1			0.1	0.2									0.3	0 - 0.6
Ceftazidime						99.7	0.1	0.1		0.1									0.3	0 - 0.6
Gentamicin						68.3	27.3	2.8	0.1	0.2	0.5	0.5	0.2						1.5	0.9 - 2.1
Tetracycline								64.1	10.7	0.1	0.5	0.3	7.9	16.6					25.2	22.9 - 27.4
Sulfamethoxazole										77.8	0.1	0.1	0.2		0.2	0.1		21.4	21.8	19.6 - 23.9
Trimethoprim					33.6	45.0	2.7						18.7						18.7	16.6 - 20.7
Ciprofloxacin	78.6	11.6	0.1	0.5	6.0	1.9	0.9			0.2	0.1								9.6	8 - 11.1
Nalidixic acid									89.3	1.4	0.3	0.1	1.2	4.0	3.8				9.0	7.5 - 10.4
Chloramphenicol										88.3	4.6	1.1	0.9	1.6	3.6				7.1	5.7 - 8.4
Azithromycin*								5.5	47.4	44.6	2.2	0.1	0.1	0.2					0.0	0 - 0.6
Colistin							99.9	0.1											0.0	0 - 0
Meropenem		99.9	0.1																0.0	0 - 0
Tigecycline					78.5	19.7	1.8												0.0	0 - 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, 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, rosé veal calves, layers and ducks in the Netherlands in 2016.

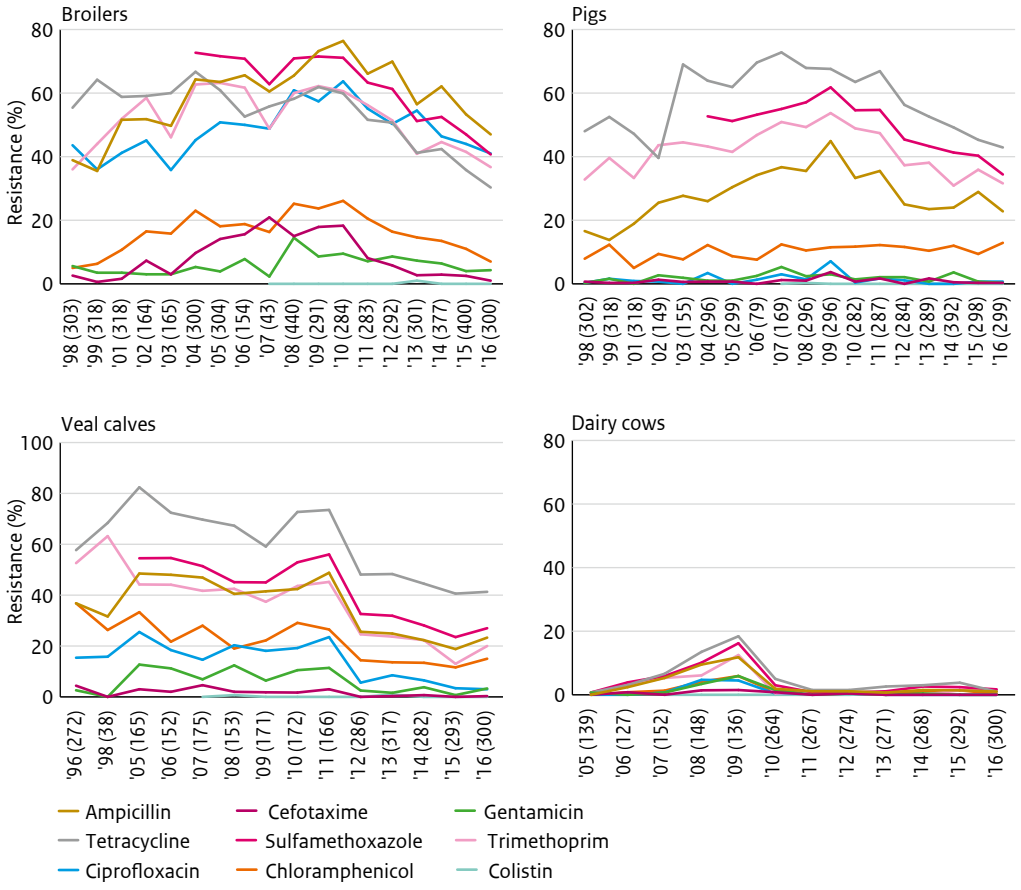
Faecal samples	Broilers	Pigs	Dairy cows	Veal calves		Layers	Ducks
	N = 300	N = 299	N = 300	White, N = 181	Rosé, N = 119	N=193	N=100
Ampicillin	47.0	23.1	1.0	31.5	10.9	5.2	8.0
Cefotaxime	1.0	0.3	0.0	0.0	0.8	0.0	0.0
Ceftazidime	1.0	0.3	0.0	0.0	0.8	0.0	0.0
Gentamicin	4.3	0.0	0.0	5.0	0.8	0.0	0.0
Tetracycline	30.3	42.8	1.0	56.4	18.5	8.8	13.0
Sulfamethoxazole	40.7	34.4	1.7	36.5	12.6	2.6	9.0
Trimethoprim	36.7	31.8	1.0	28.2	7.6	2.1	7.0
Ciprofloxacin	41.0	0.7	0.0	6.1	0.0	3.1	3.0
Nalidixic acid	39.3	0.7	0.0	4.4	0.0	3.1	0.0
Chloramphenicol	7.0	12.7	0.3	22.7	3.4	0.5	0.0
Azithromycin	0.0	0.3	0.0	2.2	0.0	0.0	0.0
Colistin	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Meropenem	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Tigecycline	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Quinolones

The highest resistance levels to quinolones were found in *E. coli* from broilers: 41.0 % resistance to ciprofloxacin and 39.3% resistance to nalidixic acid. Although resistance rates to quinolones were still high, these rates show a tendency to decrease in comparison with previous years (in 2013 for both drugs 54%; in 2014, 46 and 45%; and in 2015, 44 and 42% respectively). In 2016, high level resistance (MIC >1 mg/L) to ciprofloxacin in broilers was detected in 1.0% (3/300) of the isolates, which is a bit lower than in former years. Resistance to ciprofloxacin in 2016 was 6.1% in *E. coli* isolates from white veal calves, 3.1% in laying hens, 3.0% in ducks, remained low in slaughter pig isolates and was undetectable in isolates from rosé veal calves and dairy cows.

Resistance to quinolones in *E. coli* from meat was tested for chicken and turkey meat samples. In 2016 not only retail samples from The Netherlands (partially also from Germany or Belgium) were collected but also samples from imported meat (outside EU). Resistance levels were high to very high in chicken and turkey imported meat products. Resistance in chicken products at retail was a bit lower than in 2015: the percentage of *E. coli* with resistance to ciprofloxacin and nalidixic acid was 26.1% and 25.0%, respectively. The resistance percentages of *E. coli* in meat products were somewhat higher for ciprofloxacin than for nalidixic acid. This is probably due to the increase of plasmid mediated quinolone resistance (PMQR) exhibiting resistance to ciprofloxacin, but not to nalidixic acid.

Figure Eco01 Trends in resistance (%) of *E. coli* isolated from broilers, slaughter pigs, veal calves and dairy cattle in the Netherlands from 1998-2016.

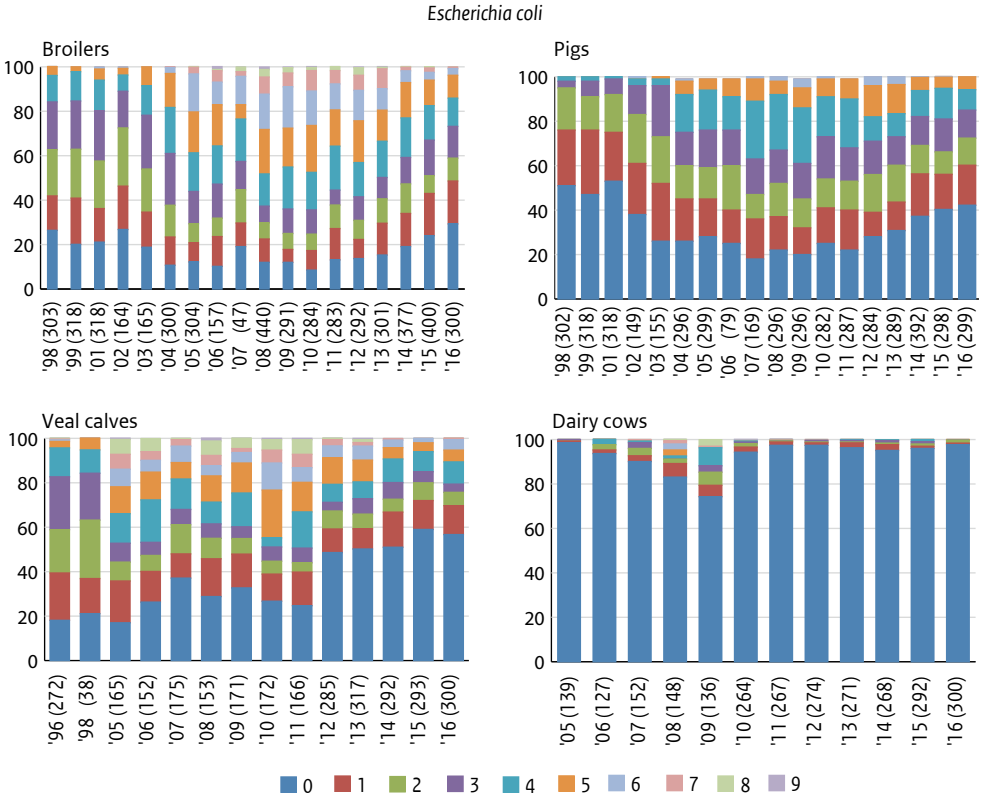


Cefotaxime resistance

Resistance to third generation cephalosporins (cefotaxime and ceftazidime), indicative of ESBL/AmpC producing *E. coli*, was detected in broilers, pigs and rosé veal calves. Cefotaxime resistance was not detected in dairy cows, white veal calves, laying hens and ducks. Resistance levels in *E. coli* were 1.0% in broilers, 0.3% in pigs and 0.8% in rosé veal calves for both, cefotaxime and ceftazidime. The 1.0% cefotaxime resistance in broilers was a further decrease in occurrence compared to 2013, 2014 and 2015 (2.7%, 2.9%, and 2.5% respectively) (Figure Eco01).

Resistance percentages to third generation cephalosporins in chicken and turkey meat samples were much higher for the imported samples, compared to the retail samples (Table Eco03). Resistance to cefotaxime in commensal *E. coli* obtained from all chicken meat samples (from import and retail) showed a slight increase, compared to 2015 (from 4.3% to 7.2%) (Figure Eco03). These figures are strongly

Figure Eco02 Resistance (%) to 0-9 antimicrobial classes among *E. coli* strains from broilers, slaughter pigs, veal calves and dairy cattle in the Netherlands from 1998-2016.



influenced by the high cefotaxime resistance of *E. coli* from imported meat (Table Eco03). Resistance to cefotaxime in all turkey meat samples decreased from 2.5% in 2015 to 1.4% in 2016. The reduction in cefotaxime resistance, determined in randomly selected *E. coli* isolates cultured on non-selective media, suggests that the concentration of *E. coli* resistant to Extended Spectrum Cephalosporins (ESC) on meat decreased. This is strengthened by the fact that the prevalence of cefotaxime resistant *E. coli* in fresh chicken meat samples using selective media decreased from 67% in 2014 to 39% in 2015 and 26.4% in 2016 (see chapter 4). The mentioned decrease of cefotaxime resistance in randomly selected *E. coli* from poultry meat is an important finding because it suggests that the exposure of humans to ESC-resistant *E. coli* through contaminated meat is reduced. In contrast, selective culturing revealed a clear and unexplained increase in the prevalence of ESBL/AmpC-producing *E. coli* in faecal samples of veal calves. The prevalence in pigs and dairy cattle also showed an increasing tendency. In broilers and layers a decrease was ESBL/AmpC-producing *E. coli* was observed (see chapter 4).

Broiler chickens

Commensal *E. coli* isolated from caecal samples from broiler chickens showed resistance to all commonly tested antimicrobials (Table Eco02). Overall, resistance levels were lower than in 2015, but level of resistance to ampicillin (47.0), tetracycline (30.3%), sulfamethoxazole (40.7%), trimethoprim (36.7%) and the quinolones ciprofloxacin (41.0%) and nalidixic acid (39.30%) remained high. Cefotaxime resistance decreased from 2.5% in 2015 to 1.0% in 2016.

Slaughter pigs

Resistance against tetracycline, sulfamethoxazole, trimethoprim and ampicillin remained high in 2016 in *E. coli* isolates from pigs and was 42.8%, 34.4%, 31.8% and 23.1%, respectively. Resistance levels of these four antibiotics showed an ongoing tendency to decrease since 2011. In 2015 a slight increase was shown for ampicillin and trimethoprim, but in 2016 resistance levels of these antibiotics were again decreased (Figure Eco01). Resistance to the 3rd generation cephalosporins was the same as in 2015 (0.3%), indicating that ESBLs are present, but in low concentrations.

Veal calves

Resistance data on white and rosé veal are reported separately. 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. In both production systems most antibiotics are administered during the starting period. On average, in white veal calves, more antibiotics are used than in rosé calves. This results in a clear difference in resistance levels between the two husbandry types. As seen in former years, a much higher resistance level was recorded for white than for 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 also the year in which the sampling strategy changed (see the description at the beginning of chapter 3.2). The changed strategy from sampling at farm to sampling at slaughterhouse might have influenced the results from 2012 and onwards. In 2016, the ratio of sampled white veal calves versus rosé veal calves changed from 50/50% to 60/40%. This ratio better reflects the proportions of slaughtered calves in The Netherlands in 2016. This explains part, but not all of the slight increase in resistant rates of *E. coli* in veal calves in 2016 compared to 2015. In 2016, resistance against the 3rd generation cephalosporins in *E. coli* isolates from white veal calves was under the detection level (TableEco02).

Dairy cattle

Resistance in *E. coli* isolated from dairy cattle is very low compared to resistance levels observed in pigs, broilers and veal calves, reflecting the low use of antibiotics in this husbandry system. Resistance rates decreased compared to 2015, and overall rates remained below 2%. No resistance to 3rd generation cephalosporins was detected.

Laying hens

In laying hens resistance percentages of *E. coli* were substantially lower than in broilers, for all antibiotics. This is most likely a result of the difference in antimicrobial usage between the two farm types. The highest resistance percentage was observed for tetracycline (8.8%). *E. coli* isolates from laying hens were not tested in 2015, but compared to 2014 resistance percentages were substantially decreased (ampicilline from 13.7% to 5.2%, tetracycline from 14.2% to 8.8%, sulfamethoxazole from 5.8% to 2.6% and trimethoprim from 5.8% to 2.1%).

Ducks

There are no historical data available on antibiotic usage in ducks. Table Eco02 shows that in *E. coli* isolated from ducks resistance was observed for the same antimicrobials as in laying hens, but resistance percentages were a bit higher. Highest resistance percentages were measured for tetracycline, sulfamethoxazole, ampicilline and trimethoprim (13.0%, 9.0%. 8.0% and 7.0% respectively).

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. The data with the determined level of multidrug resistance over the years are shown in Figure Eco02.

The data from 2016 indicate a decreasing trend in the level of multidrug resistance in broilers and pigs, but a slight increase in veal calves. The increase in calves might have been caused by the changed ratio in samples from white and rosé calves (see before). However, levels of multidrug resistance (resistant to three or more classes of antibiotics) remained still quite high among *E. coli* originating from broilers (41.0%), pigs (27.6%) and veal calves (24.3%). In dairy cattle multidrug resistance in *E. coli* again was rare with only 0.3% (1 out of 300) of the isolates showing resistance to three or more classes of antimicrobials.

The overall increasing tendency of the number of completely susceptible *E. coli* isolates, especially in broilers and pigs (Figure Eco02), is ongoing and might be the best indicator to reflect the long term effect of the more prudent use of antibiotics on the level of multidrug resistance in the intestinal flora.

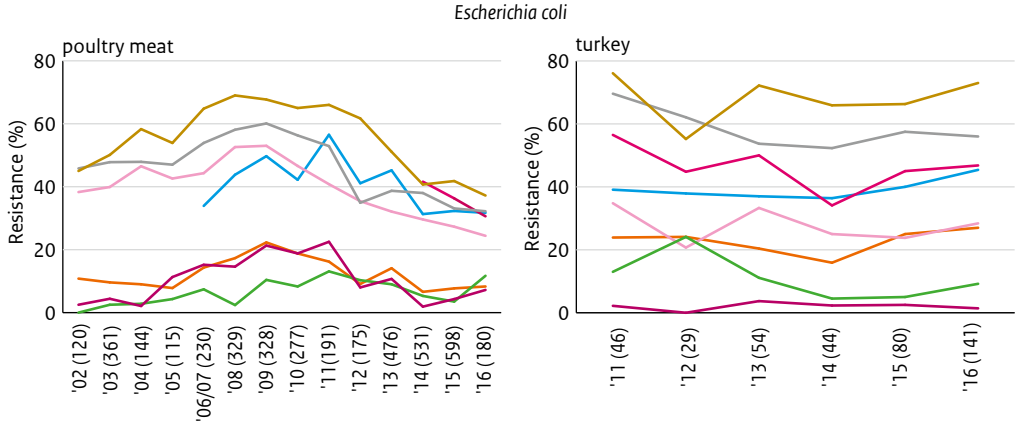
3.2.2 *E. coli* in raw meat products of food-animals

Resistance percentages of *E. coli* isolated from raw meat products from chicken and turkey, sampled at retail and from import products by the Dutch Food and Consumer Product Safety Authority (NVWA), are shown in Table Eco03. The trends in resistance are presented in Fig Eco03. The resistance rates in trends in resistance of isolates from chicken and turkey meat are for the first time given separately for import products and products in retail (which can include meat produced in The Netherlands, but also other EU countries). After a tendency to decrease from 2010 to 2014, resistance rates in chicken meat products seem to have stabilized or even increased in 2015 and 2016. In turkey meat, resistance rates have been at a constant high level since 2011. Cefotaxime resistance in *E. coli* isolates from chicken products showed, after a rapid decrease from 10.7% in 2013 to 1.9% in 2014, a slight increase to 4.3% in 2015, and a further increase to 7.2% in 2016. Fluctuations in the resistance rates might be caused by year-to-year differences in the proportion of foreign chicken and turkey products included in the survey.

Table Eco03 Resistance (in %) of *E. coli* isolated from raw chicken and turkey meat products in the Netherlands in 2016.

Meat products	Chicken import	Chicken retail	Turkey import	Turkey retail
	N = 46	N = 134	N = 9	N = 132
Ampicillin	63.0	28.4	55.6	74.2
Cefotaxime	21.7	2.2	11.1	1.5
Ceftazidime	19.6	1.5	11.1	1.5
Gentamicin	39.1	2.2	11.1	9.1
Tetracycline	50.0	26.1	66.7	55.3
Sulfamethoxazole	58.7	20.9	55.6	46.2
Trimethoprim	45.7	17.2	33.3	28.0
Ciprofloxacin	58.7	22.4	88.9	42.4
Nalidixic acid	43.5	20.9	55.6	28.0
Chloramphenicol	21.7	3.7	66.7	24.2
Azithromycin	6.5	0.0	0.0	4.5
Colistin	0.0	0.7	0.0	8.3
Meropenem	0.0	0.0	0.0	0.0
Tigecycline	0.0	0.0	0.0	0.0

Figure Eco03 Trends in resistance (%) of *E. coli* isolated from raw chicken and turkey meat products in the Netherlands from 1998 - 2016.



3.2.3 *Enterococcus faecalis* and *E. faecium*

In this chapter, information on resistance in *Enterococcus* species, as indicator organism for the occurrence and trends in resistance in Gram-positive bacteria from food-producing animals in the Netherlands, is presented. From 2013 onwards, as a result of less priority for including enterococci in the surveillance, poultry, pigs and cattle and meat thereof were sampled once every three years. From 2016, no enterococci from faecal samples were tested, but in 2016 *Enterococcus faecalis* and *E. faecium* were isolated from chicken and turkey meat samples. The poultry meat samples were taken at retail.

Highlights

1. In chicken meat, highest resistance levels were observed for erythromycin (55.4% for *E. faecalis* and 57.1% for *E. faecium*) and tetracycline (66.1% and 25.0% respectively). In addition, a high level of resistance was observed for quinu/dalfopristin in *E. faecium* (42.9%).
2. In turkey meat, highest resistance levels were observed for erythromycin (65.1% for *E. faecalis* and 58.8% for *E. faecium*) and tetracycline (88.9% and 76.5% respectively). A high resistance percentage was also observed for quinu/dalfopristin in *E. faecium* (58.8%).

Resistance levels

In 2016 resistance rates have been determined for 56 *E. faecalis* and 28 *E. faecium* strains isolated from chicken meat samples as well as for 63 *E. faecalis* and 17 *E. faecium* isolates from turkey meat samples. Resistance percentages for *E. faecalis* and *E. faecium* isolated from these products are presented in Table Ento1. Trends over the years in chicken meat are shown in Figure Ento1.

Chicken meat

High resistance levels in *E. faecalis* as well as in *E. faecium* were observed for erythromycin (55.4% and 57.1%) and tetracycline (66.1% and 25.0%), (Table Ento1). In *E. faecium*, a traditionally high level of resistance was observed for quinu/dalfopristin (42.9%), and low levels for ampicillin (7.1%) and daptomycin (14.3%) . Figure Ento1 shows a slight increase in resistance level for erythromycin since 2013 (no data for 2014 and 2015) in both enterococci species, and a decrease in resistance level for tetracycline. For daptomycin no data from earlier years were available for comparison. The resistance percentage for ciprofloxacin in *E. faecium* showed a substantial decrease since 2013 (from 23.6% to 7.1%).

Turkey meat

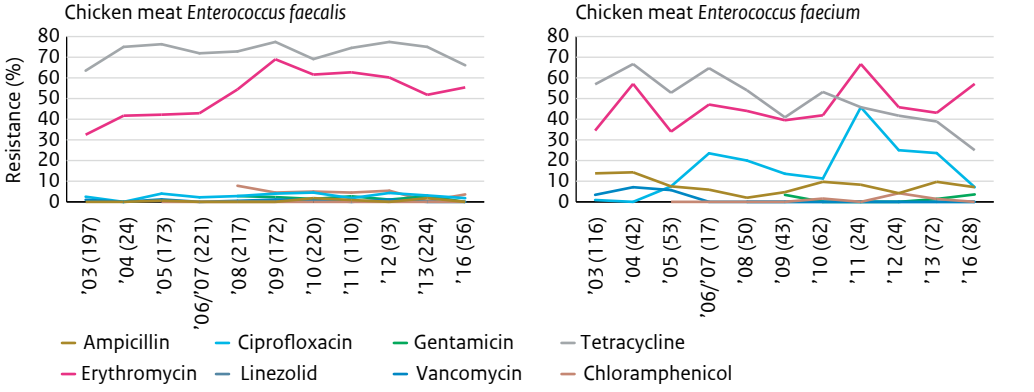
As in the chicken meat samples, also in the turkey meat samples high resistance levels were observed for erythromycin (65.1% and 58.8%) and tetracycline (88.9% and 76.5%), (Table Ento1). Also for these samples, a high resistance percentage was seen for quinu/dalfopristin in *E. faecium* (58.8%), and a lower level for ampicillin in *E. faecium* (17.6%). The data for *E. faecium* might be not representative, because of the low number of isolates (n=17).

Table Ent01 Resistance % of *Enterococcus faecalis* and *E. faecium* strains isolated from raw chicken and turkey meat in the Netherlands in 2016.

	Chicken meat		Turkey meat	
	E. faecalis (N = 56)	*E. faecium (N = 28)*	*E. faecalis (N = 63)*	*E. faecium (N = 17)*
Ampicillin	0.0	7.1	0.0	17.6
Chloramphenicol	3.6	0.0	7.9	0.0
Ciprofloxacin	1.8	7.1	4.8	0.0
Daptomycin	0.0	14.3	0.0	0.0
Erythromycin	55.4	57.1	65.1	58.8
Gentamicin	0.0	3.6	1.6	0.0
Linozelid	0.0	0.0	0.0	0.0
Quinu/dalfopristin*	-	42.9	-	58.8
Teicoplanin	0.0	0.0	0.0	0.0
Tetracycline	66.1	25.0	88.9	76.5
Tigecycline	0.0	0.0	0.0	0.0
Vancomycin	0.0	0.0	0.0	0.0

* *E. faecalis is intrinsic resistant to quinu/dalfopristin*

Figure Ent01 Trends in resistance percentages of *Enterococcus faecium* and *E. faecalis* isolated from veal calves in the Netherlands from 1998-2016.



4
Screening for ESBL, AmpC, carbapenemase-producing and colistin-resistant Enterobacteriaceae in food-producing animals and meat in the Netherlands in 2016

Highlights

1. ESBL/AmpC-producing *Escherichia coli* represented 0.3% of the randomly isolated *E. coli*, the lowest proportion observed since 2007.
2. In spite of the above, selective culturing in livestock faeces indicated that the prevalence (% of animal carriers) of ESBL/AmpC-producing *E. coli* marked a general tendency to increase in livestock, excluding broilers and layers. Currently an explanation for this phenomenon is lacking.
3. A follow up of the 2009 study on within-farm prevalence of ESBL/AmpC-producing *E. coli* in broilers showed a significant decrease from 66% in 2009 to 38% in 2016.
4. The proportion of fresh chicken meat with ESBL/AmpC-producing *E. coli* isolates decreased to 24% (67% in 2014, 39.4% in 2015). In imported chicken meat the proportion was much higher (61.2%).
5. The most prevalent ESBL/AmpC gene in *E. coli* from livestock and meat was $bla_{\text{CTX-M-1}}$ in almost all animal species followed by $bla_{\text{CMY-2}}$, $bla_{\text{SHV-12}}$, $bla_{\text{TEM-52}}$ and $bla_{\text{CTX-M-14}}$.
6. The prevalence of ESBL/AmpC-producing *Salmonella* in 2016 was 1.7%, confirming the decreasing trend observed in the period 2013–2015. Most represented ESBL/AmpC genes were $bla_{\text{CMY-2}}$, generally associated with *S.* Saintpaul, $bla_{\text{CTX-M-14b}}$ in *S.* Kentucky, and $bla_{\text{CTX-M-9}}$ in S. Typhimurium.
7. The majority of ESBL-producing *Salmonella* isolates from humans were highly multidrug resistant, with most of the isolates showing a resistant phenotype to 5-8 antibiotics (67%).
8. No carbapenemase-producing *Enterobacteriaceae* were detected in in livestock and companion animals.
9. The colistin resistance gene *mcr-1* was present at low level in *E. coli* from livestock (0.5%) and in retail meat from turkeys (8.3%) and chicken (0.7%).

4.1 ESBL/AmpC-producing bacteria

4.1.1 Randomly isolated ESBL/AmpC-producing bacteria from livestock in 2016

Surveillance of resistance to extended spectrum cephalosporins in the Netherlands is routinely done by random isolation of a minimum of 170 isolated *E. coli*, each representing one epidemiological unit, from faecal samples of food-producing animals as prescribed by EFSA guidelines.[1] These isolates are tested for susceptibility to cefotaxime and ceftazidime. Proportions of resistant isolates are determined based on EUCAST epidemiological cut-off values as described in Chapter 3. Since 1998, cefotaxime resistance was observed at low levels in all animal species. Figure ESBL01 shows the percentage of cefotaxime resistance in randomly picked *E. coli* isolated from non-selective media derived from broilers, slaughter pigs (1998 – 2016), veal calves and dairy cows (2005 – 2016). In broilers, after 2003 an apparent increase

[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.

in cefotaxime resistance was observed up to levels that varied between 15 – 20%, with the highest peak observed in 2007. The prevalence in broilers steadily declined to 2.7% in 2013, to reach a minimum of 1% in 2016. The strong decline observed in 2011, from 18.3% to 8.1%, was most likely the result of decreased usage of antibiotics in broilers since the spring of 2010 when the (off label) use of ceftiofur was ceased at Dutch hatcheries. In 2014, the decrease in usage stopped in broilers, which resulted in the levelling off observed in 2015 and the lowest registered prevalence so far in 2016.
From a total of 1492 randomly selected *E. coli* isolates that were tested in 2016, five displayed reduced susceptibility (MIC > 0.25 mg/L) to cefotaxime (see also 3.2.1). Three were isolated from broilers, one from a slaughter pig and one from a veal calf (Table ESBL01). In dairy cows no ESBL/AmpC-suspected *E. coli* isolates were found in 2016. Cefotaxime resistant isolates were screened for beta-lactamase gene families using PCR or the Check-Points CT101 miniaturised micro-array. Subsequently the genes were identified by dedicated PCR and sequence analysis. All isolates with a negative array result for ESBL or AmpC genes were examined for promoter mutants in the chromosomal *ampC* genes. The results of this molecular typing are displayed in Table ESBL01.
In broiler isolates three plasmid meditated ESBL/AmpC genes were present: $bla_{CTX\text{-}M\text{-}1}$, $bla_{TEM\text{-}52c}$, and $bla_{CMY\text{-}2}$. 2016 is the second year after 2015 in which $bla_{TEM\text{-}52c}$ was not found in cefotaxime resistant isolates from broilers derived from the monitoring program. $bla_{CTX\text{-}M\text{-}1}$ was also detected in a rosé veal calf isolate. Mutation in the chromosomal *ampC* gene was detected in one of the pig isolates (*ampC*-type 18). Variants of $bla_{CTX\text{-}M\text{-}14}$ (CTX-M-9 group) were not detected in 2016.
It can be concluded that by random isolation, only three plasmid mediated ESBL/AmpC genes were found in 1492 isolates in 2016 (0.3%), the lowest prevalence observed since 2007. This confirms the already promising results of 2015, when 0.9% ESBL/AmpC-producing isolates where detected, a major improvement compared to 2009 when ESBL/AmpC-producing isolates added up to 7.6%, before antibiotic usage reduction started in Dutch livestock.

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

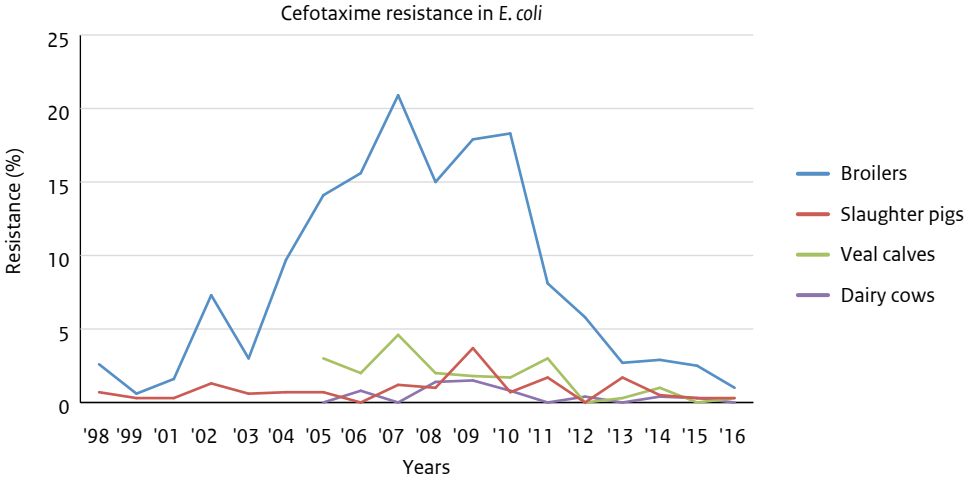


Table ESBL01 ESBL-genes found in *E. coli* isolates with reduced susceptibility to cefotaxime derived from broilers, veal calves, slaughter pigs, dairy cows and turkey (only 2011 and 2012) during 2007-2016.

	ESBLs isolated from						ESBL-genes detected											
Year	Broilers	Veal calves	Slaughter pigs	Dairy cows	Turkeys	Total ESBL suspected (n)	CTX-M-1-group#	CTX-M-2	CTX-M-9-group!	TEM-52c	TEM-20	SHV-12*	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
Total	266	27	31	8	6	338	133	19	3	38	2	42	9	44	20	28		

 # All were $bla_{CTX-M-1}$, only in 2011 one $bla_{CTX-M-3}$ gene was found in an isolate from veal calves.
Three combinations (all in broiler isolates) 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}.

** One combination of bla_{SHV-12} together with bla_{TEM-52} occured in 2012 in one broiler isolate.*

n.t. : not tested

Selective isolation of ESBLs in 2016

As of 2014 an active surveillance by selective culturing for ESBL/AmpC-producers in broilers was implemented together with the ongoing active surveillance in pigs and veal calves that started in 2011. Faecal samples taken for monitoring at slaughterhouse (slaughter pigs, white and rosé veal calves, broilers, layers, and ducks) and at farms (dairy cows) were also used for ESBL/AmpC-producing *E. coli* detection by selective methods. 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. This resulted in the screening of 1500 faecal samples (Table ESBL02).

In 2016, also 1395 meat samples (Table ESBL04) were analysed for ESBL/AmpC-producing *E. coli*. 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 *Enterobacteriaceae* morphology were selected for bacterial species identification, and confirmed *E. coli* were analysed for ESBL/AmpC-genes presence and screened for beta-lactamase gene families, as described above.

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

The prevalence of ESBL/AmpC-producing *E. coli* in faecal samples is shown in Table ESBL02. Suspected ESBL/AmpC isolates comprised all *E. coli* growing on MacConkey with 1 mg/L cefotaxime, including ESBL/AmpC negative isolates as well as isolates carrying mutations in the chromosomal *ampC* gene promoter. Confirmed ESBL isolates included only ESBL or AmpC gene-carrying isolates, most likely located on a horizontally transmissible plasmid. Each sample represented one slaughter batch of animals from one farm. Of the 1500 samples analysed for ESBL/AmpC-producing *E. coli*, 26.9% were positive, mainly due to the high prevalence in broilers (50.3%). A surprising increase in prevalence was observed in both white and rosé veal calves (33.9% and 28.7%, respectively) compared to 2015 (17.3% and 10%, respectively). As already noted in the past, ESBL/AmpC-producing *E. coli* levels in white veal calves were higher than in rosé veal calves. The slight reduction observed in ESBL/AmpC-producing *E. coli* in broilers in 2015 was confirmed also in 2016 (from 56.5% to 50.3%). Similar results were observed for layers, where ESBL/AmpC-producing *E. coli* prevalence dropped from 32.5% in 2014 to 28% in 2016. Prevalence in pigs was increased compared to 2015 (from 12.3% to 16.3%), as well as in dairy cows (from 9.3% to 13.2%). ESBL/AmpC-producing *E. coli* prevalence in ducks attested to 13%; the absence of previous data for this animal species does not allow for a comparison in time. In conclusion, 2016 marked a slight tendency to increase of ESBL/AmpC-producing *E. coli* carriership in livestock, excluding broilers and layers. An explanation for this phenomenon is not available yet.

ESBL/AmpC genes detected in animal faeces are reported in Table ESBL03. The increase in ESBL types variation observed in 2014 and 2015 compared to former years (MARAN 2011 and 2013) was confirmed in 2016, likely a consequence of the new surveillance method implemented in 2014, with a collection of faecal samples derived from a minimum of 150 to 400 different farms per animal species (MARAN 2014). Like in former years, $bla_{CTX\text{-}M\text{-}1}$ was the dominant ESBL-variant in all animal species examined (n=202 out of 367 genes), followed by $bla_{CMY\text{-}2}$ (n=52), $bla_{SHV\text{-}12}$ (n=31) and $bla_{TEM\text{-}52c}$ (n=30). Two $bla_{CTX\text{-}M\text{-}2}$ gene variants were reported in slaughter pig and white veal calf for the first time since 2014. The low variation in ESBL-types observed in broilers in the randomly isolated *E. coli* (Table ESBL01) mirrored the results of the selective culturing with 7 ESBL gene types compared to 2015. The increased ESBL/AmpC-producing *E. coli* prevalence observed in veal calves was associated with the highest gene variability (12 different ESBL genes). The more classical human associated genes $bla_{CTX\text{-}M\text{-}9}$, $bla_{CTX\text{-}M\text{-}14}$, and $bla_{CTX\text{-}M\text{-}15}$ were described in veal calves, conversely to 2015 where they were predominant in broilers. A similar phenomenon was observed in dairy cows, where increased prevalence in isolates matched an increase in ESBL-types with $bla_{CTX\text{-}M\text{-}1}$ being the predominant beta-lactamase gene. $bla_{CTX\text{-}M\text{-}55}$ was detected in dairy cows for the first time since gene typing was performed (MARAN 2011). Slaughter pig and layer hen isolates didn't show significant differences compared to previous years. Chromosomal *ampC* types confirmed their growing role in conferring cefotaxime resistance as already observed in 2015 with relatively high numbers in pig, layer, and dairy cow isolates (19%, 10%, and 13%, respectively). Conversely from previous years, no combination of ESBL gene types within the same isolate was detected.

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 2016.

	N samples	N suspected ESBL	N confirmed ESBL	Prevalence(%) ESBL confirmed
Broilers	300	151	151	50.3
Layers	193	60	54	28.0
Ducks	100	13	13	13.0
Pigs	300	61	49	16.3
Veal calves				
white	183	64	62	33.9
rosé	122	35	35	28.7
Dairy cows	302	46	40	13.2
Total	1500	430	404	26.9

Table ESBL03 Beta-lactamases identified in *E. coli* from broilers, slaughter pigs, veal calves and dairy cows in 2016. Data derived from the active surveillance of ESBL-producing *E. coli* at slaughter.

		Broilers	Layers	Ducks	Slaughter pigs	Veal calves White	Veal calves Rose	Dairy cows	Total
CTX-M-1 group									
	CTX-M-1	66	33	4	35	31	14	19	202
	CTX-M-15	3				15	5	7	30
	CTX-M-32					1	4	5	10
	CTX-M-55	2	2			1	1	1	7
CTX-M-2 group									
	CTX-M-2				1	1		2	4
CTX-M-8/25 group									
	CTX-M-8				1				1
	CTX-M-9						1		1
CTX-M-9group									
	CTX-M-14		3		3	2	6	2	16
	CTX-M-27					1			1
	CTX-M-65			1		3	1	2	7
TEM									
	TEM-52c	19		1	5	3	1	1	30
	TEM-52cVar	6	3		2				12
	TEM-225		1						1
SHV									
	SHV-12	27				2	1	1	31
CMY									
	CMY-2	28	12	7	2	2	1		52
Chromosomal ampC									
	ampC-type-3		6		12	2		4	24
	ampC-type-3-like							2	
Total		235			56	28	15	33	367

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

Prevalence of ESBL suspected isolates in fresh raw meat are shown in Table ESBL04. Meat preparations (except for imported frozen poultry meat with approximately 1% salt) were not screened in 2016. Out of 1395 fresh and import meat samples, 133 were tested positive for ESBL/AmpC-producing *E. coli* (9.5%) and six samples were tested positive for *E. coli* with chromosomal mutations in the AmpC promotor region. The decreasing trend observed in imported poultry meat since 2012 (83%) and continued in the past two years (67% and 60% in 2014 and 2015, respectively) was confirmed in 2016, with a prevalence of 61.2% in imported chicken meat. Turkey meat showed a decrease in ESBL/AmpC-producing *E. coli* prevalence depending on the source, with 15% prevalence in fresh meat (22.5% in 2015) and 62.5% in imported meat (66.7% in 2015). While cattle and lamb meat showed ESBL/AmpC prevalence comparable to 2015 (between 2.0% and 2.7%) incidence in fresh calf meat was higher than 2014 and 2015 (from 0% to 4.4%). The first year of fresh goat meat sampling revealed a relatively high ESBL/AmpC-producing *E. coli* prevalence (7.7%).

All 139 isolates were selected for molecular typing and confirmed by MALDI-TOF as *E. coli*. Table ESBL05 shows the different ESBL/AmpC gene types detected in meat. Most of ESBL/AmpC genes found in beef and veal were also found in faecal samples of veal calves ($bla_{CTX\text{-}M\text{-}1}$, $bla_{CTX\text{-}M\text{-}15}$, $bla_{CTX\text{-}M\text{-}55}$, and $bla_{CMY\text{-}2}$) strongly suggesting faecal contamination during slaughter and/or meat processing. Chicken meat displayed more ESBL/AmpC gene variability than broiler faecal samples, with $bla_{CTX\text{-}M\text{-}2}$ and $bla_{CTX\text{-}M\text{-}8}$ not detected in the latter. Chromosomal *ampC* types were detected mainly in turkey meat isolates, together with a great variety of ESBL gene types. The dominant human $bla_{CTX\text{-}M\text{-}15}$ was not detected in chicken meat although it was detected in broiler faecal samples (Table ESBL03). Other frequent ESBL/AmpC gene types were $bla_{CMY\text{-}2}$ and $bla_{SHV\text{-}12}$ typically found in respective livestock, with an increase in $bla_{CTX\text{-}M\text{-}2}$ and $bla_{CTX\text{-}M\text{-}8}$ detection compared to 2015.

Table ESBL04 Prevalence of ESBL/AmpC-positive *E. coli* isolates from raw meat in the Netherlands in 2016.

Animal source		N screened	N ESBL/AmpC suspected	N ESBL/AmpC tested at WBVR	N ESBL/AmpC positive	% ESBL/AmpC positive
Beef						
	fresh meat	299	7	5	5	2.0
Veal						
	fresh meat	205	11	9	9	4.4
Pork						
	fresh meat	273	1	0	0	n.d.
Chicken						
	fresh meat	208	55	51	50	24.0
	import	49	32	30	30	61.2
Turkey						
	fresh meat	187	35	33	28	15.0
	import	8	5	5	5	62.5
Lamb						
	fresh meat	112	3	3	3	2.7
Sheep						
	fresh meat	28	1	1	1	3.6
Goat						
	fresh meat	26	2	2	2	7.7
Total		1395	152	139	133	9.5

Table ESBL05 Beta-lactamases identified in *E. coli* from raw meat products in the Netherlands in 2016.

	ESBL gene	Chicken	Turkey	Beef	Veal	Lamb	Sheep	Goat	Totaal
CTX-M-1 group									
	CTX-M-1	21	8	2	2	1		2	36
	CTX-M-3		1						1
	CTX-M-15		3	1	4				8
	CTX-M-32	1							1
	CTX-M-55	3	1		2				6
CTX-M-2 group									
	CTX-M-2	7							7
CTX-M-8/25 group									
	CTX-M-8	4	5						9
CTX-M-9 group									
	CTX-M-14		1						1
	CTX-M-27		1						1
	CTX-M-65		1						1
TEM									
	TEM-52c	2	5						7
	TEM-52cVar	3							3
SHV									
	SHV-12	6	5						11
CMY									
	CMY-2	33	2	2	1	2	1		41
Chromosomal ampC									
	ampC-type-3		3						3
	ampC-type-5		2						2
	ampC-type-11	1							1
Total		81	38	5	9	3	1	2	139

Chicken: 30 isolates were derived from imported frozen meat and 50 from fresh retail meat.
Turkey: 5 isolates were derived from frozen imported meat and 34 isolates from retail meat.

ESBL/AmpC-producing *Salmonella*

Surveillance of resistance to extended spectrum cephalosporins is also done in *Salmonella enterica* isolated in the Netherlands. In 2016 a selection of 2089 *Salmonella* isolates sent to RIVM for sero- or MLVA-typing were tested for susceptibility to cefotaxime and ceftazidime. In addition, NVWA tested 135 *Salmonella* mainly obtained from raw meat. In total, cefotaxime resistant *Salmonella* were isolated in 36 samples mainly from humans (n=26), poultry (n=6), and turkey (n=4) from which 35 isolates were further typed (Table ESBL06). The prevalence of ESBL/AmpC-producing *Salmonella* was 1.7%, confirming the decreasing trend observed in 2014 and 2015 (2.1% and 1.9%, respectively) and almost half of 2013 (4%). The predominance of *S.* Heidelberg observed in 2015 was not confirmed in 2016, as *S.* Kentucky, which is known to originate from Northern Africa, was the most prevalent (n=9), followed by Infantis, Saintpaul, Typhimurium and six other serovars identified to carry ESBL/AmpC genes (material and methods are the same as described above for *E. coli*. One *S.* Minnesota isolate from poultry meat was not included in the molecular analysis.

ESBL/AmpC genes detected in *Salmonella* are reported in Table ESBL06. The most represented genes were: i) $bla_{\text{CMY-2}}$, generally associated with *S.* Saintpaul and Heidelberg and also present in 2 other serovars; ii) $bla_{\text{CTX-M-14b}}$ in *S.* Kentucky; and iii) $bla_{\text{CTX-M-9}}$ in *S.* Typhimurium. Compared to previous years, prevalence of $bla_{\text{CMY-2}}$ kept dropping from 58% (2014) to 35% (2015) to 28% (2016). Similarly, $bla_{\text{CTX-M-1}}$ and $bla_{\text{CTX-M-15}}$ were less represented. $bla_{\text{CTX-M-9}}$ and $bla_{\text{CTX-M-14b}}$ appeared to be highly predominant compared to previous years with an increase from 1-6% to 11-25%, respectively. No ESBL/AmpC gene combination was detected. In isolates from human sources a variety of ESBL/AmpC genes were found including $bla_{\text{CTX-M-55}}$, $bla_{\text{CTX-M-65}}$, $bla_{\text{CTX-M-1}}$, and $bla_{\text{CTX-M-14}}$.

All cefotaxime resistant *Salmonella* isolates were highly multidrug resistant, as shown in Table ESBL07. The increased finding of multi-resistance observed in 2015 compared to 2014 (70% vs 23%) was confirmed in 2016 with most of the isolates being resistant to 5 - 8 antibiotics (67%). 3% of the isolates were resistant to 9 out of 10 antibiotics, but no resistance was detected against meropenem or azithromycin. Colistin resistance observed in 8.8% of isolates in 2015 dropped to 0% in 2016.

ESBL/AmpC gene types found in *Salmonella* since 2007 are summarized in Table ESBL08. Every year genes $bla_{\text{CMY-2}}$, $bla_{\text{TEM-52}}$, and those belonging to the $bla_{\text{CTX-M-1}}$-group have been found in *Salmonella* isolates from diverse sources. After detection in 2015, $bla_{\text{CTX-M-2}}$ was not detected in 2016. $bla_{\text{DHA-1}}$ was identified for the first time in a human isolate of *S.* Bovismorbificans. Overall, *Salmonella* isolates held less variability in ESBL/AmpC gene types than 2015.

In conclusion, ESBL/AmpC-producing *E. coli* are widespread in Dutch food-producing animals and in raw meat mainly of poultry origin. ESBL/AmpC- was 0.3% of the randomly isolated *E. coli* , the lowest observed since 2007. Selective culturing in faecal samples of food-producing animals showed a slight tendency to increase of animals carrying ESBL/AmpCs for veal calves compared to 2015.

The dominant ESBL/AmpC gene types were confirmed to be $bla_{\text{CTX-M-1}}$ and $bla_{\text{CMY-2}}$, in all animal species independent of the source of isolation, whereas an increased detection of $bla_{\text{CTX-M-14}}$ was registered in both *E. coli* and *Salmonella*. The dominant human ESBL gene $bla_{\text{CTX-M-15}}$ was frequently found in veal calves and dairy cows faecal samples as well as in beef and veal samples. $bla_{\text{CTX-M-15}}$ was only rarely found in broilers and it was absent in chicken products (Table ESBL06), as already observed in 2015.

Table ESBL06 Beta-lactamases in *Salmonella* isolated in 2016

				CTX-M-1 group			CTX-M-9 group			TEM	CMY	DHA	
Serovar	Humans	Poultry	Turkey	CTX-M-1	CTX-M-15	CTX-M-55	CTX-M-9	CTX-M-14b	CTX-M-65	TEM-52Var	CMY-2	DHA-1	Total
1,4,5,12:i:-	3					3							3
Bovismorbificans	1											1	1
Bredeney	2										2		2
Heidelberg	1	2									3		3
Infantis	4	1		3					2				5
Kentucky	9							9					9
Paratyphi B variant Java	1	2			1					2			3
Saintpaul			4								4		4
Thompson	1										1		1
Typhimurium	4						4						4
Total	**26**	**5**	**4**	**3**	**1**	**3**	**4**	**9**	**2**	**2**	**10**	**1**	**35**

This table contains the results of seven extra Salmonella isolates derived from turkey meat (S. Saintpaul, N = 4) and poultry meat (S. Heidelberg, N = 2, S. Paratyphi variant Java, N = 1) at NVWA.
One ESBL-suspected isolate from poultry meat (S. Minnesota) collected at NVWA was not included in the analysis. previous years.

Table ESBL07 Resistance and multidrug resistance percentages of ESBL-producing *Salmonella* in the Netherlands in 2016.

Antimicrobials	R%
Ampicillin	100.0
Cefotaxime	100.0
Ceftazidime	88.9
Gentamicin	47.2
Tetracycline	69.4
Sulfamethoxazole	75.0
Trimethoprim	38.9
Ciprofloxacin	86.1
Nalidixic acid	63.9
Chloramphenicol	25.0
Azithromycin	0.0
Colistine	0.0
Meropenem	0.0
Tigecycline	5.6

Multi drug resistance	N = 35
0	0%
1	0%
2	3%
3	22%
4	6%
5	11%
6	31%
7	11%
8	14%
9	3%
10	0%

4.1.2 Decreased prevalence of ESBL/AmpC-producing *E. coli* in broilers parallel to a reduced usage of antimicrobials in the Netherlands

In 2009 a study on prevalence of ESBL/AmpC producing *E. coli* on Dutch broiler farms showed that all broiler farms included in the study (n=26) were positive for ESBL/AmpC-producing *E. coli* (Dierikx *et al,* 2013). The within-farm prevalence (based on 41 faecal samples) appeared to be >80% (for 85% of the farms) and >90% (62% of the farms). Antimicrobial use in animals has been drastically reduced from 2009 to 2016. In the routine national surveillance program a decline in ESBL/AmpC-producing *E. coli* in broilers was observed. In 2016 this study was repeated on the same farms with the aim to describe the prevalence of ESBL/AmpC-producing *E. coli* and compare with the results from 2009.

All 26 farms that were included in 2009 were asked to participate again. Farms were visited twice during a production cycle (at start and just before slaughter). From each house on the farm 41 cloacal swabs were collected and analysed by enrichment in LB broth supplemented with 1 mg/L cefotaxime and subsequent inoculation on MacConkey agar supplemented with 1 mg/L cefotaxime for the presence of ESBL/AmpC-producing *E. coli*. Ten % of the strains was analysed for the presence of ESBL/AmpC genes by PCR. PCR-positive isolates were sequenced for ESBL/AmpC allele variant identification. Information about cleaning and disinfection, farm management and antimicrobial treatment was collected on each farm.

In total, 20 of the original 26 farms agreed to participate again. For all comparative analyses, only the farms from 2009 that also participated in 2016 were selected. The differences in farm management between 2009 and 2016 were small. Most important was the transition towards the slower growing Hubbard-line (4% to 25%). Regarding antimicrobial use 62% (2009) of the farms vs 15% (2016) had an early treatment of chickens and 4% (2009) vs 60% (2016) did not use antimicrobials for the sampled flock. The proportion of farms on which animals with ESBL/AmpCs were found just before slaughter remained high: 100% in 2009 to 95% in 2016. The within-farm prevalence decreased significantly from 66% of the animals in 2009 (range: 24-100%) to 38% (range: 0-98%) in 2016. Remarkable is the fact that on farms, at the same locations, houses were present with and without detected ESBL/AmpC-producing *E. coli*. There was no significant difference in prevalence between farms without antimicrobial treatment (36%) and farms with one or more treatments (42%). Typing showed that bla_{CMY-2}, bla_{CTX-M-}1 and bla_{SHV-12} were the most prevalent types both in 2009 and 2016. Within farms and within flocks different ESBL/AmpC variants were detected at the same time of sampling.

In summary, the within-farm prevalence of ESBL/AmpC-producing *E. coli* in broilers decreased significantly from 66% in 2009 to 38% in 2016, parallel to a huge reduction in antimicrobial use on these farms. Given this reduction in prevalence, in 2016 a differentiation between high and low prevalent farms could be made, in contrast with high prevalent farms only in 2009. However, despite the reduced numbers of carriers found in 2016 compared to 2009, ESBL/AmpC-producing *E. coli* is still widespread in the Dutch broiler production industry. It can be concluded that risk factors should be investigated at house level instead of farm level. Finally, the diversity of ESBL/AmpC types within flocks suggests common driver and not the presence of one successful clone.

Table ESBL08 ESBL-genes found in *Salmonella* isolates displaying reduced susceptibility to cefotaxime during 2007-2016

Year	CTX-M-1-group#	CTX-M-2##	CTX-M-8	CTX-M-9-group*	TEM-52	TEM-20	SHV-12**	CMY-2***	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	7			15	2			10		1	36	2117	1.7
Total	100	45	8	35	47	4	10	123	3	1	375	17784	2.1

 # 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).

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

** 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).*

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

**** 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*

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

4.2 Carbapenemases

4.2.1 Monitoring of carbapenemase producing Enterobacteriaceae in livestock

In 2015 a sensitive method was applied to screen for carbapenemase producers, extended spectrum beta-lactamases that can also hydrolyse carbapenems. This is important in an environment with a very low anticipated prevalence of carbapenem resistance. All faecal samples sent to the Wageningen Bioveterinary Research (WBVR) by the Dutch Food and Consumer Protection Authority (NVWA) for antimicrobial resistance surveillance were screened with this method. Samples were grown overnight in Buffered Peptone Water (BPW). After incubation the culture was centrifuged and DNA isolated from pellet. A commercial RT-PCR (Check-Points, CarbaCheck MDR RT) which 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 on commercial selective plates (ChromID CARBA and ChromID OXA (Biomerieux) and on HIS plates with 0.125 mg/L ertapenem (for *Shewanella*).

Carbapenemase screening in 2016 (n=1800) resulted in two bla_{OXA-48}-like positive samples in the RT-PCR (one slaughter pig and one veal calf faecal samples). bla_{OXA-48}-like genes are known to be chromosomally associated with *Shewanella* spp. 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 and 2015 (MARAN 2016). Considering that *Shewanella* spp. is the natural progenitor of this carbapenemase family (Zong, 2012), carrying bla_{OXA-48}-like genes on the chromosome, 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 and in food products will continue in 2017, to monitor potential carbapenemase gene spread among environmental and clinically relevant bacteria.

4.2.1 Monitoring of carbapenemase producing Enterobacteriaceae in companion animals

Within Europe, carbapenemase producing *Enterobacteriaceae* (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). *So* far, in the Netherlands CPE have not been detected. In order to detect introduction of CPE, a monitoring for CPE in Dutch companion animals was initiated in 2015. The screening for CPE comprised of a retrospective and a prospective study.

In the retrospective study, clinical isolates were obtained through the Veterinary Microbiological Diagnostic Center (VMDC) of Utrecht University. Since CPE are frequently reported in combination with ESBLs, all available ESBL-suspected isolates stored since 2009 were included in the screening. ESBL-suspicion was based on susceptibility to 3rd generation cephalosporins (ceftiofur). In total, 418 isolates were screened, originating from dogs (n=281), cats (n=73), horses (n=49), cattle (n=9) and other animal species (n=6). The isolates were obtained from divers matrices, including urine (47%), wound/abdominal fluid (22%) or 'other' (32%; e.g. pus, tracheal swab/lavage, horse uterus secrete). All isolates were screened using a disk diffusion assay (imipenem: 10 mg, ertapenem: 10 mg, meropenem: 10 mg) and results were interpreted as described by Cohen-Stuart *et al*, 2010. Suspected carbapenemase-producing strains were screened by PCR for bla_{KPC}, bla_{NDM}, bla_{VIM}, bla_{IMP} and bla_{OXA-48}. All screened isolates were negative for CPE.

In the prospective study, faecal samples of cats and dogs were screened for the presence of CPE. The inclusion criterion for dogs was antimicrobial treatment in the week prior to sampling or at the moment of sampling. For collection of samples and selection of patients, dermatology and internal medicine specialists from 4 referral clinics participated. Additional faecal samples that met the antimicrobial treatment criterion were obtained through VMDC. Since cats are not frequently treated with antimicrobials, no inclusion criterion was given. All available faecal samples from cats submitted to VMDC were included. In 2015, 201 and 101 faecal samples from cats and dogs, respectively, were screened. In 2016, 178 and 145 faecal samples from cats and dogs were screened, respectively. From each sample, 0.5 gram feces were suspended in 4.5 ml TSB broth, supplemented with 50 mg/L vancomycin. After enrichment, the suspension was inoculated on ChromID Carba-Smart agar (BioMerieux). In addition, DNA of the enrichment broth was isolated for molecular screening using the RT-PCR Check-MDR carba kit (Check-Points). All screened faecal samples were negative for CPE.

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 indicator *E. coli* from faecal samples from 2014 and 2015 indicated a decreasing trend in the occurrence of this gene. To gain more knowledge on the current spread of *mcr-1* in livestock, a prospective study was performed in 2016 as part of the national surveillance program on antibiotic resistance in animals to reveal the current spread of this gene in livestock. For this purpose purified DNA of pooled BPW cultures (five samples per pool) from a total of 1500 faecal samples were tested with conventional PCR for the presence of *mcr-1* 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 4 mg/L colistin. As a result *mcr-1* positive *E. coli* were identified in eight faecal samples (0.5%) in different animal species: veal calves (n=4), broilers (n=2), pig (n=1) and dairy cow (n=1). In 2016, no colistin resistant *E. coli* isolates were identified amongst the indicator *E. coli* isolated from 1500 faecal samples. However, in meat fourteen colistin resistant *E. coli* non-selectively isolated from retail meat were confirmed as *mcr-1* carriers. These isolates almost exclusively originated from turkey (n=11) and chicken meat (n=2). The remaining *mcr-1*-positive isolate was obtained from imported crocodile 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 in raw meat from chicken and turkey, but not in *Salmonella*.

References

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