

**Centrum voor Onderzoek in Diergeneeskunde en Agrochemie
Centre d'Etude et des Recherches Vétérinaires et Agrochimiques**



CODA - CERVA

Antimicrobial resistance

in *Salmonella* species

from poultry in 2016 in Belgium

Report on the occurrence of antimicrobial resistance in *Salmonella* species from poultry in 2016 in Belgium.

Summary

Salmonella spp. isolates were obtained in the context of the national *Salmonella* control programme in the primary production in Belgium. *Salmonella* serovar-specific data displayed large variability at the antimicrobial resistance level, with some serovars exhibiting greater resistance to certain antimicrobials or expressing multidrug resistance to a higher degree than other serovars. In *Salmonella* spp. from laying hens, antimicrobial resistance was absent, whereas in 2015, resistance to colistin was associated with *S. Enteritidis*. In broiler chickens however, antimicrobial resistance against sulfamethoxazole, ciprofloxacin, nalidixic acid, tetracycline, ampicillin and trimethoprim increased as compared to 2015. Antimicrobial resistance to colistin declined. Random sampling from non-selective culture plates didn't detect any *Salmonella* spp. resistant to cefotaxime and ceftazidime. In broiler chickens, *S. Infantis* was predominantly present and its presence even increased as compared to previous year, whereas in laying hens, *S. Enteritidis* was by far the most predominant serovar isolated. Levels of resistance were generally highest for *S. Infantis*, followed by *S. Typhimurium* isolated from broiler chickens. Multi-resistance, defined as resistance to at least three different antimicrobial classes, was seen in 50% of the *S. Infantis* strains. Co-resistance to ciprofloxacin, nalidixic acid and to sulfamethoxazole was a frequently recurring phenotypic resistance pattern. The horizontally transferable colistin resistance gene *mcr-1* or *-2* was not detected in phenotypically resistant *Salmonella* *Enteritidis*. No carbapenemase producing *Salmonella* were found. Clinically relevant levels of tigecycline resistance were present in 2015 in *Salmonella* from broiler chickens, but were not present this year.

Introduction

Salmonella spp. is one of the most important bacterial zoonotic agents. In spite of a decrease in the number of *Salmonella* spp. related infections, salmonellosis continues to be the second most commonly reported zoonotic disease in Belgium, as well as in the entire European Union (EU) (EFSA and ECDC, 2015). In Belgium, for instance, 3119 human cases of salmonellosis were reported in 2015 (FAVV, personal communication). Belgium has made efforts to reduce the prevalence of *Salmonella* spp. in flocks of breeder and broiler chickens, laying hens and meat turkeys by the implementation of a national control and monitoring programme. In view of its zoonotic aspect, antimicrobial susceptibility surveillance of *Salmonella* spp. is of great importance, as acquired resistance can hamper treatment of infected humans. Also, antimicrobial resistance in *Salmonella* spp. may be located on transferable elements and therefore take part in the spread of resistance to commensal and pathogenic human- and animal-related pathogens. The monitoring of zoonotic agents and its related antimicrobial resistance became mandatory for EU member states by the implementation of Directive 2003/99/EC and Regulation (EC) No 2160/2003, assuring comparability of data. The specific monitoring of AMR of isolates from layers, broilers and meat turkeys in the framework of the national *Salmonella* control programmes is laid down in Commission decision 2013/652/EU.

In this report, antimicrobial susceptibility data are presented for *Salmonella* spp. isolated from food-producing animals, more precisely from broiler chickens and laying hens. Antimicrobial susceptibility data on *Salmonella* spp. isolated from animal species other than poultry were not included in the monitoring program for 2016. Antimicrobial resistance data of *Salmonella* spp. isolated from food products from animals, as potential sources for distribution to humans via the food chain, are reported by the Institute for Public Health (WIV-ISP).

Materials and methods

Sampling

All *Salmonella* spp. isolates were obtained in the context of the national *Salmonella* control programmes organised by the Federal Agency for the Safety of the Food Chain FASFC - www.favv.be) and were analysed at CODA-CERVA (Veterinary and Agrochemical Research center, the National Reference Laboratory for antimicrobial resistance in animal productions). In addition, most *Salmonella* spp. that were isolated for diagnostic reasons or strains obtained during field research were also sent to the reference laboratory for serotyping. Most *Salmonella* spp. isolates were sent in by the regional laboratories (*Dierengezondheidszorg Vlaanderen* [www.dgz.be] and *Association Régionale de Santé et d'Identification Animales* [www.arsia.be]) and by other veterinary laboratories recognized by the FASFC which are involved in the official monitoring programmes.

Isolation and identification

Salmonella spp. was isolated at several laboratories (DGZ, ARSIA, laboratories of the Federal Food Agency, Lavetan, ...) using the ISO 6579:2002/Amd1:2007 Annex D method (ISO, 2007). Serotyping was performed at CODA-CERVA (Veterinary and Agrochemical Research center, the National Reference Laboratory for antimicrobial resistance), according to the Kauffman-White-Le Minor scheme (Grimont and Weill, 2007; Guibordenche et al., 2010).

Antimicrobial susceptibility testing

Antimicrobial susceptibility of *Salmonella* spp. strains was tested using a micro broth dilution method (Trek Diagnostics). To this end, 1 to 3 colonies were suspended in sterile physiological water to an optical density of 0.5 McFarland. Ten microliter of this suspension is inoculated in 11 ml cation adjusted Mueller Hinton broth with TES buffer.

Fifty microliter of the Mueller-Hinton broth with bacteria was brought on a micro-titer plate with the antimicrobials lyophilised, produced by Trek Diagnostics, using the auto-inoculating system of Trek Diagnostics. The antimicrobial substances incorporated in the antimicrobial susceptibility testing were recommended by the European Food Safety Agency (EFSA) and included in the decision 2013/652/EU of the Commission. They were selected based on their public health relevance and as representatives of different antimicrobial classes (EFSA, 2012). Table 1 shows the antimicrobial substances tested, their abbreviations, the dilutions used and the epidemiological cut-off's (ECOFFs), established by the European Committee on Antimicrobial Susceptibility (EUCAST) or as defined by the EU reference laboratory on antimicrobial resistance (DTU) (EUCAST, 2017).

Plates were incubated 18-24 hours at 35°C and read. The Minimal Inhibitory Concentration (MIC) was defined as the lowest concentration by which no visible growth could be detected. MICs were semi-automatically recorded by the Trek Vision system using the SWIN software. Results were automatically exported to an Excel file.

Table 1 : Panel of antimicrobial substances included in antimicrobial susceptibility testing, EUCAST epidemiological cut-off's (ECOFFs), and concentration ranges tested in *Salmonella* spp.

Antimicrobial (Abbreviation)	Concentration range, mg/l	<i>Salmonella</i> EUCAST ECOFF
Ampicillin (AMP)	1–64	> 8
Cefotaxime (FOT)	0.25–4	> 0.5
Ceftazidime (TAZ)	0.5–8	> 2
Meropenem (MERO)	0.03–16	> 0.125
Nalidixic acid (NAL)	4–128	> 16
Ciprofloxacin (CIP)	0.015–8	> 0.064
Tetracycline (TET)	2–64	> 8
Colistin (COL)	1–16	> 2
Gentamicin (GEN)	0.5–32	> 2
Trimethoprim (TMP)	0.25–32	> 2
Sulfamethoxazole (SMX)	8–1024	NA ^(a)
Chloramphenicol (CHL)	8–128	> 16
Azithromycin (AZI)	2–64	NA ^(b)
Tigecycline (TGC)	0.25–8	> 1

EUCAST: European Committee on Antimicrobial Susceptibility Testing

NA: not available.

(a): > 256 mg/l was used

(b): > 16 mg/l was used

The co-resistance patterns

In *Salmonella* spp. isolates, co-resistance to cefotaxime (FOT) and ciprofloxacin (CIP) was estimated, as these two antimicrobials are of particular interest in human medicine. Co-resistance was addressed using both ECOFFs (FOT > 0.5 mg/l and CIP > 0.064 mg/l) and CBPs (FOT > 2 mg/l and CIP > 0.06 mg/l) for *Salmonella* spp., established by EUCAST or as defined by the EU reference laboratory for antimicrobial resistance (DTU) (EUCAST, 2017).

Data description

In this report, an overview of antimicrobial resistance prevalence data is given for all isolated *Salmonella* serovars in broiler chickens and laying hens. Particular attention is given to the occurrence of antimicrobial resistance for selected *Salmonella* serovars of public health importance, based on the prevalence data of the Scientific Institute of Public Health (*S. Typhimurium* and its monophasic variants, *S. Enteritidis*, *S. Kentucky*, *S. Infantis*, *S. Derby*, *S. Brandenburg*, *S. Virchow*, *S. Oranienburg*, *S. Agona* and *S. Stanley*) (WIV-ISP, 2013).

Throughout the report, terms used to describe the levels or occurrence of antimicrobial resistance are those proposed by EFSA. 'Rare: <0.1 %', 'very low: >0.1 % to 1.0 %', 'low: >1 % to 10.0 %', 'moderate: >10.0 % to 20.0 %', 'high: >20.0 % to 50.0 %', 'very high: >50.0 % to 70.0 %', 'extremely high: >70.0 %'.

A multi-resistant isolate is defined as resistant to at least three different antimicrobial substances, belonging to antimicrobial classes represented by the antimicrobials included in the analysis (Table 1). Resistance to nalidixic acid and resistance to ciprofloxacin, as well as the resistance cefotaxime and ceftazidime are respectively addressed together when considering multi-resistance. The frequency and percentage of isolates exhibiting multi-resistance were determined for the most prevalent *Salmonella* serovars in broiler chickens and laying hens, as well as for *Salmonella* serovars of public health importance (*S. Typhimurium* and its monophasic variants, *S. Enteritidis*, *S. Kentucky*, *S. Infantis*, *S. Derby*, *S. Brandenburg*, *S. Virchow*, *S. Oranienburg*, *S. Agona* and *S. Stanley*).

Results

Overall, 167 *Salmonella* spp. were isolated from broiler chickens (n= 127) and laying hens (n= 39). A summary of the *Salmonella* serotyping results is presented for broiler chickens (Table 2) and laying hens (Figure 4). Antimicrobial resistance profiles varied considerably among animal categories and among recovered *Salmonella* serovars (Table 2).

Broiler chickens

Antimicrobial resistance

Antimicrobial resistance prevalences for *Salmonella* spp. from broiler chickens (n= 127) are represented in Figure 1. Highest levels of resistance were reported for sulfamethoxazole, ciprofloxacin, nalidixic acid, tetracycline, ampicillin and trimethoprim. Antimicrobial resistance to these antimicrobials increased compared to 2015. Only low levels were seen for chloramphenicol, gentamicin and colistin. Antimicrobial resistance to colistin clearly declined compared to previous year. *Salmonella* spp. was fully susceptible to azithromycin, cefotaxime, meropenem, ceftazidime and tigecycline.

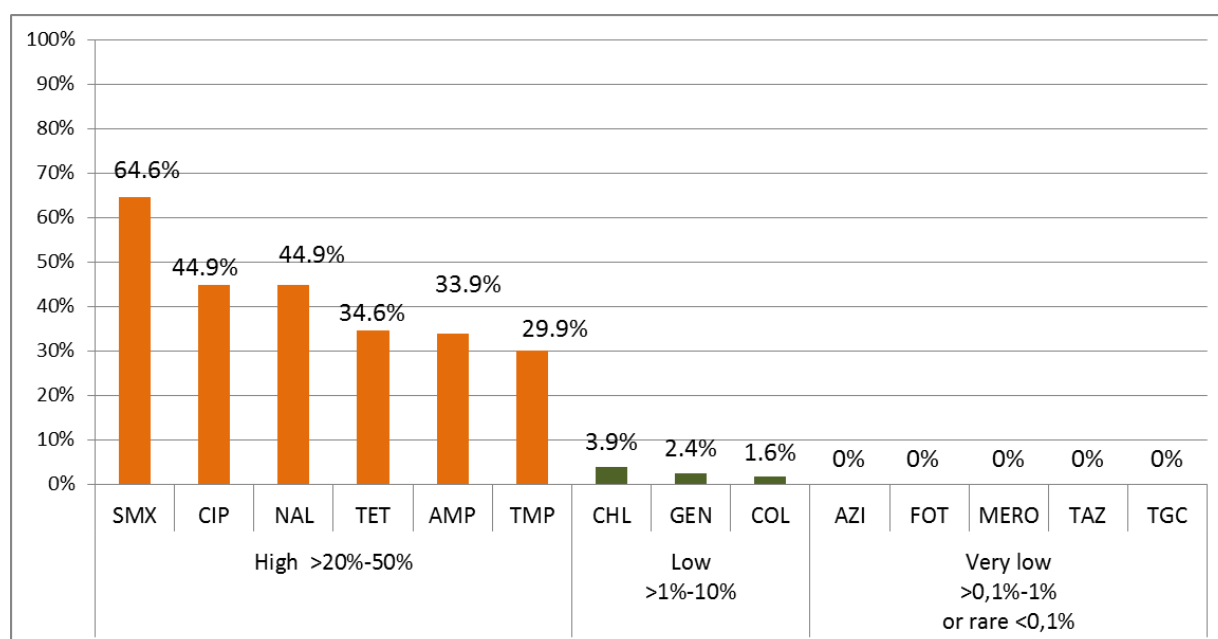


Figure 1 : Antimicrobial resistance prevalence for *Salmonella* spp. (n= 127), isolated from broiler chickens, based on epidemiological cut-off's, according to the European Committee on Antimicrobial Susceptibility (EUCAST) for ampicillin (AMP), azithromycin (AZI), chloramphenicol (CHL), ciprofloxacin (CIP), colistin (COL), cefotaxime (FOT), gentamicin (GEN), meropenem (MERO), nalidixic acid (NAL), sulfamethoxazole (SMX), ceftazidime (TAZ), tetracycline (TET), tigecycline (TGC) and trimethoprim (TMP).

In broiler chickens, 26.0% of *Salmonella* spp. were fully susceptible and 40.9% showed multi-resistance (resistance to at least three different antimicrobial classes). There is clearly less full susceptibility and more multi-resistance than the previous year (47.0% full susceptibility and 20.9% multi-resistance). Resistance to 2 antimicrobials was most frequently observed (26.0%), followed by multi-resistance to 3 different antimicrobial classes (17.3%) (Figures 2 and 3). Resistance to 4 or 5 antimicrobial classes was present in 13.4% and 10.2% respectively, represented by *S. Infantis*, *S. Typhimurium* O5- and O5+, *S. Paratyphi* B, and *S. 4,12:i:-*.

The relative contribution of different *Salmonella* serovars in the total number of *Salmonella* spp. strains isolated from broiler chickens and their antimicrobial resistance prevalence can be found in Table 2. *S. Infantis* was predominantly present in broiler chickens (n= 52, 40.9%). Its presence has strongly increased since previous year (n= 27, 20.1%). *S. Infantis* was only fully susceptible in 3 out of the 52 strains (5.8%). Antimicrobial resistance to ampicillin, tetracycline and trimethoprim was higher than in 2015. Tigecycline resistance was no longer present, whereas 3 *S. Infantis* strains were

resistant to tigecycline in 2015. Antimicrobial resistance to 2 different antimicrobial classes occurred most frequently (n= 22, 42.3%). Co-resistance to ciprofloxacin, nalidixic acid and to sulfamethoxazole was a recurring phenotypic resistance pattern (22 out of 52 *S. Infantis* strains) and resistance to these antimicrobials was higher than previous year. Multi-resistance to 3, 4 or 5 different classes was seen in 19.2%, 7.7% and 23.1% of the *S. Infantis* strains (Figures 2 and 3). Co-resistance to sulfamethoxazole, tetracycline, quinolones, trimethoprim and ampicillin was present.

S. Typhimurium O5- and O5+ (n= 17, 13.4%) and *S. Gaminara* (n= 14, 11.0%) were also frequently isolated (Table 2). Other *Salmonella* serovars of public health importance were prevalent as follows: *S. Typhimurium* O5+ (n= 6, 4.7%) and its monophasic variant (n= 5, 3.9%), *S. Agona* (n=2, 1.6%), *S. Enteritidis* (n= 2, 1.6%) and *S. Derby* (n= 3, 2.4%). *S. Brandenburg*, *S. Kentucky*, *S. Oranienburg*, *S. Stanley* and *S. Virchow* were not isolated.

Table 2 : Antimicrobial resistance prevalence for *Salmonella* serovars isolated from broiler chickens (n= 127), based on epidemiological cut-off's, according to the European Committee on Antimicrobial Susceptibility (EUCAST) for ampicillin (AMP), azithromycin (AZI), chloramphenicol (CHL), ciprofloxacin (CIP), colistin (COL), cefotaxime (FOT), gentamicin (GEN), meropenem (MERO), nalidixic acid (NAL), sulfamethoxazole (SMX), ceftazidime (TAZ), tetracycline (TET), tigecycline (TGC) and trimethoprim (TMP).

<i>Salmonella</i> spp. n= 127 (%)	AMP	AZI	CHL	CIP	COL	FOT	GEN	MERO	NAL	SMX	TAZ	TET	TGC	TMP
<i>S. Infantis</i> 52 (40.9%)	15 28.9%	0 0%	0 0.0%	48 92.3%	0 0%	0 0%	0 0.0%	0 0.0%	48 92.3%	48 92.3%	0 0%	24 46.2%	0 0%	16 30.8%
<i>S. Typhimurium</i> O5- and O5+ 23 (18.1%)	18 78.3%	0 0.0%	5 21.7%	6 26.1%	0 0.0%	0 0.0%	0 0.0%	0 0.0%	6 26.1%	14 60.9%	0 0.0%	13 56.5%	0 0.0%	10 52.2%
<i>S. Gaminara</i> 14 (11.0%)	0 0.0%	0 0.0%	0 0.0%	0 0.0%	0 0.0%	0 0.0%	1 7.1%	0 0.0%	0 0.0%	2 14.3%	0 0.0%	0 0.0%	0 0.0%	0 0.0%
<i>S. Paratyphi</i> B 6 (4.7%)	1 16.7%	0 0.0%	0 0.0%	1 16.7%	0 0.0%	0 0.0%	0 0.0%	0 0.0%	1 16.7%	6 100%	0 0.0%	0 0.0%	0 0.0%	6 100%
<i>S. Livingstone</i> 5 (3.9%)	0 0.0%	0 0.0%	0 0.0%	0 0.0%	0 0.0%	0 0.0%	0 0.0%	0 0.0%	0 0.0%	0 0.0%	0 0.0%	0 0.0%	0 0.0%	0 0.0%
<i>S. 4,5,12:i:-</i> 5 (3.9%)	5 100%	0 0.0%	0 0.0%	0 0.0%	0 0.0%	0 0.0%	0 0.0%	0 0.0%	0 0.0%	5 100%	0 0.0%	5 100%	0 0.0%	0 0.0%
<i>S. Mbandaka</i> 4 (3.1%)	0 0.0%	0 0.0%	0 0.0%	1 25.0%	0 0.0%	0 0.0%	2 50.0%	0 0.0%	1 25.0%	2 50.0%	0 0.0%	1 25.0%	0 0.0%	0 0.0%
<i>S. Derby</i> 3 (2.4%)	3 100%	0 0.0%	0 0.0%	0 0.0%	0 0.0%	0 0.0%	0 0.0%	0 0.0%	0 0.0%	3 100%	0 0.0%	0 0.0%	0 0.0%	3 100%
<i>S. Agona</i> 2 (1.6%)	0 0.0%	0 0.0%	0 0.0%	0 0.0%	0 0.0%	0 0.0%	0 0.0%	0 0.0%	0 0.0%	1 50.0%	0 0.0%	1 50.0%	0 0.0%	0 0.0%
<i>S. Enteritidis</i> 2 (1.6%)	0 0.0%	0 0.0%	0 0.0%	0 0.0%	2 100%	0 0.0%	0 0.0%	0 0.0%	0 0.0%	0 0.0%	0 0.0%	0 0.0%	0 0.0%	0 0.0%
<i>S. Rissen</i> 1 (0.8%)	0 0.0%	0 0.0%	0 0.0%	0 0.0%	0 0.0%	0 0.0%	0 0.0%	0 0.0%	0 0.0%	0 0.0%	0 0.0%	0 0.0%	0 0.0%	0 0.0%
<i>S. Senftenberg</i> 1 (0.8%)	0 0.0%	0 0.0%	0 0.0%	1 100%	0 0.0%	0 0.0%	0 0.0%	0 0.0%	1 100%	0 0.0%	0 0.0%	0 0.0%	0 0.0%	0 0.0%
<i>S. Colorado</i> 1 (0.8%)	0 0.0%	0 0.0%	0 0.0%	0 0.0%	0 0.0%	0 0.0%	0 0.0%	0 0.0%	0 0.0%	0 0.0%	0 0.0%	0 0.0%	0 0.0%	0 0.0%
<i>S. Idikan</i> 1 (0.8%)	0 0.0%	0 0.0%	0 0.0%	0 0.0%	0 0.0%	0 0.0%	0 0.0%	0 0.0%	0 0.0%	0 0.0%	0 0.0%	0 0.0%	0 0.0%	0 0.0%
<i>S. Indiana</i> 1 (0.8%)	0 0.0%	0 0.0%	0 0.0%	0 0.0%	0 0.0%	0 0.0%	0 0.0%	0 0.0%	0 0.0%	0 0.0%	0 0.0%	0 0.0%	0 0.0%	0 0.0%
<i>S. Kottbus</i> 1 (0.8%)	0 0.0%	0 0.0%	0 0.0%	0 0.0%	0 0.0%	0 0.0%	0 0.0%	0 0.0%	0 0.0%	0 0.0%	0 0.0%	0 0.0%	0 0.0%	0 0.0%
<i>S. Llandof</i> 1 (0.8%)	0 0.0%	0 0.0%	0 0.0%	0 0.0%	0 0.0%	0 0.0%	0 0.0%	0 0.0%	0 0.0%	0 0.0%	0 0.0%	0 0.0%	0 0.0%	0 0.0%
<i>S. 4,12:i:-</i> 1 (0.8%)	1 100%	0 0.0%	0 0.0%	0 0.0%	0 0.0%	0 0.0%	0 0.0%	0 0.0%	0 0.0%	1 100%	0 0.0%	1 100%	0 0.0%	1 100%
<i>S. 6,7:z29r:-</i> 1 (0.8%)	0 0.0%	0 0.0%	0 0.0%	0 0.0%	0 0.0%	0 0.0%	0 0.0%	0 0.0%	0 0.0%	0 0.0%	0 0.0%	0 0.0%	0 0.0%	0 0.0%

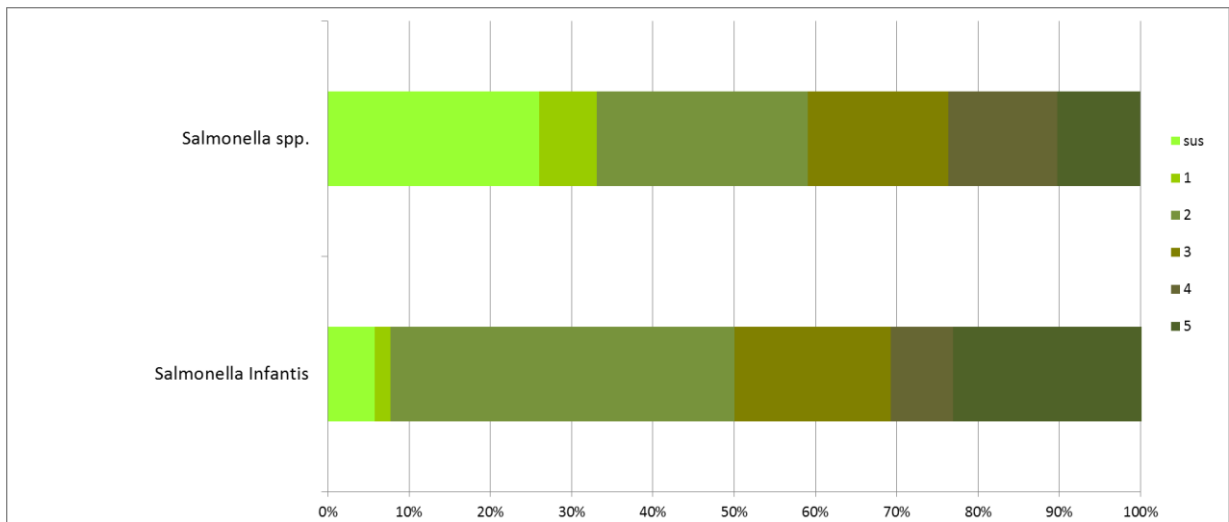


Figure 2 : Percentages of *Salmonella* serovars (n= 127) and *S. Infantis* (n= 52) from broiler chickens showing full susceptibility (“sus”) or resistance to at least 1 antimicrobial. Resistance to nalidixic acid and resistance to ciprofloxacin, as well as the resistance to cefotaxime and ceftazidime are respectively addressed together.

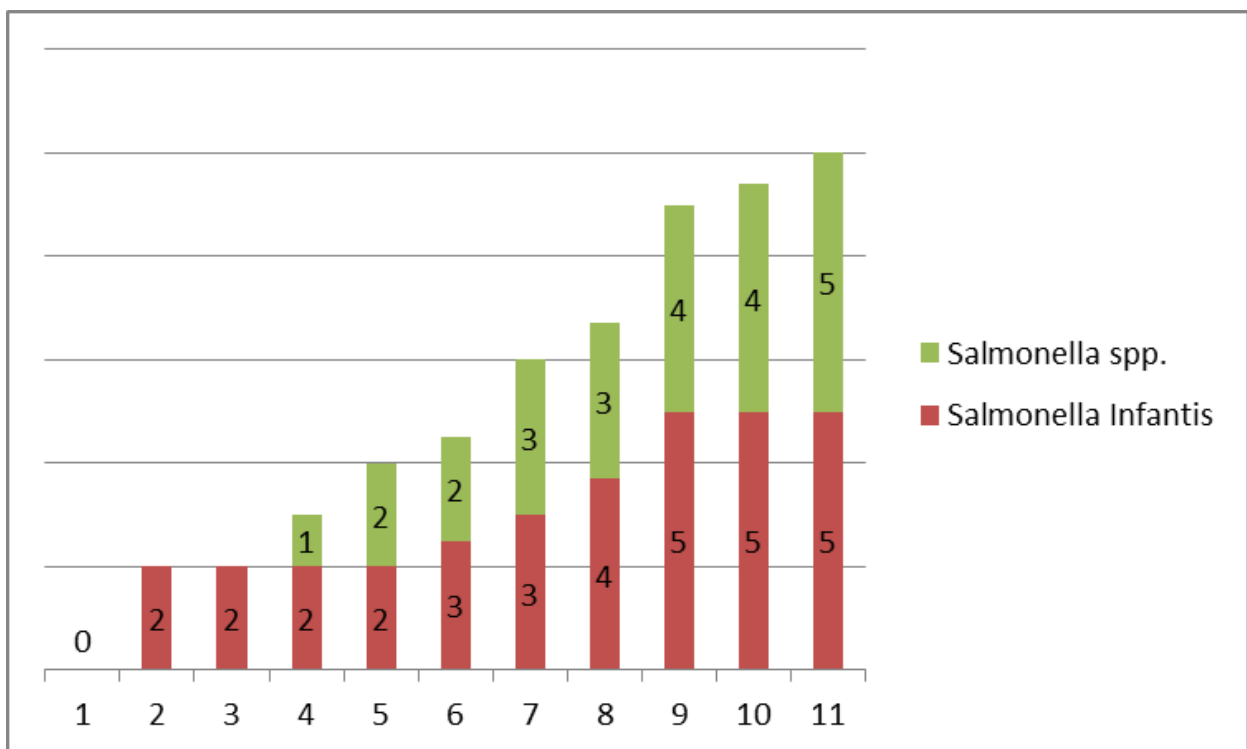


Figure 3 : Percentiles of all *Salmonella* serovars (n= 127) and *S. Infantis* (n= 52) from broiler chickens showing full susceptibility (“sus”) or resistance to at least 1 antimicrobial. Resistance to nalidixic acid and resistance to ciprofloxacin, as well as the resistance to cefotaxime and ceftazidime are respectively addressed together.

Co-resistance to cefotaxime and ciprofloxacin in *Salmonella* spp.

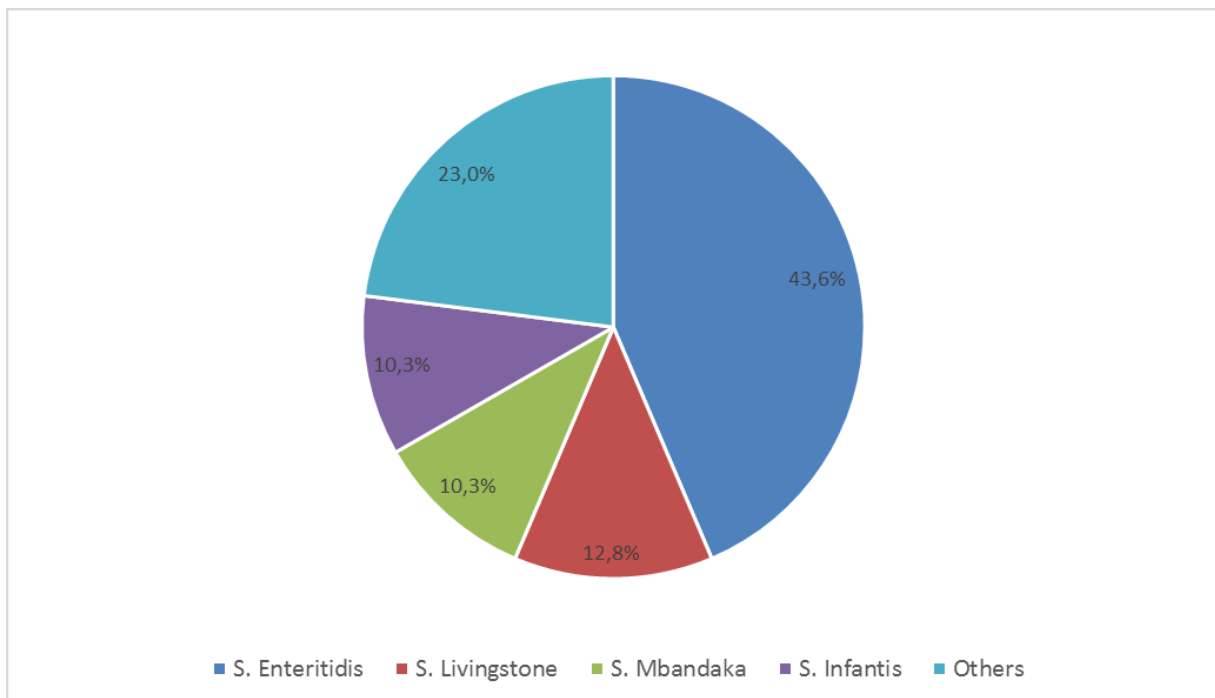
None of the *Salmonella* spp. strains showed co-resistance to cefotaxime and ciprofloxacin, based on epidemiological cut-off values or clinical breakpoints.

Laying hens

Antimicrobial resistance

In laying hens, all 39 *Salmonella* spp. strains were fully susceptible. In 2015, still 40.0% of the *Salmonella* isolates showed colistin resistance, which could almost entirely be attributed to *Salmonella* Enteritidis. Antimicrobial resistance to ciprofloxacin and nalidixic acid was found in one strain in 2015, but was also absent this year.

The relative contribution of different *Salmonella* serovars in the total number of *Salmonella* spp. strains isolated from laying hens can be found in Figure 4. *S. Enteritidis* was by far the most predominant serovar isolated from laying hens (n= 17, 43.6%), whereas other serovars were less frequently isolated. Other *Salmonella* serovars of public health importance were prevalent as follows: *S. Infantis* (n= 4, 10.3%). *S. Agona*, *S. Brandenburg*, *S. Derby*, *S. Typhimurium* and its monophasic variant, *S. Kentucky*, *S. Oranienburg*, *S. Stanley* and *S. Virchow* were not isolated.



Figuur 4 : The relative contribution of the *Salmonella* serovars isolated from laying hens (total number of strains= 39).

Co-resistance to cefotaxime and ciprofloxacin in *Salmonella* spp.

None of the *Salmonella* spp. strains showed co-resistance to cefotaxime and ciprofloxacin, based on epidemiological cut-off values or clinical breakpoints.

Discussion

The monitoring of *Salmonella* spp. prevalence in food-producing animals, potential sources of human salmonellosis, is a mandatory programme established by the European Commission. Temporal trends in the occurrence of *Salmonella* spp. in food-producing animals can be consulted in the annual reports of CODA-CERVA.

Salmonella serovar-specific data displayed large variability at the antimicrobial resistance level, with some serovars exhibiting greater resistance to certain antimicrobials or expressing multidrug resistance to a higher degree than other serovars. For some serovars only low numbers were isolated and serovar-specific prevalence of antibiotic resistance should therefore be nuanced.

Antimicrobial resistance in *Salmonella* spp. showed higher levels for broiler chickens compared to 2015, whereas for laying hens, all *Salmonella* spp. strains were fully susceptible. This is remarkable as for the laying hens, in 2015, considerable resistance to colistin in *S. Enteritidis* was present. *S. Enteritidis* has been reported with an intrinsic lower susceptibility towards colistin of an unknown genetic nature (Agersø et al., 2012). Indeed, in 2015, *S. Enteritidis* from broiler chickens and laying hens showed increased MIC values of 4 or 8 µg/ml (epidemiological cut-off value = 2 µg/ml) (CODA-CERVA, 2016). Also, this year, the two *S. Enteritidis* strains from broiler chickens displayed decreased susceptibility to colistin (MIC= 4 µg/ml), but MIC values ≥ 4 µg/ml for colistin were absent in *S. Enteritidis* from laying hens. No other serovars displayed colistin resistance. Until recently, colistin resistance was only described as the consequence of chromosomally located mutations. The existence of horizontally transferable resistance genes (*mcr-1* and *mcr-2*) have now globally been reported in bacteria from animals, food and humans (Arcilla et al., 2015; Hasman et al., 2015; Liu et al., 2015; Webb et al., 2015, Xavier et al., 2016). In Belgium, both *mcr-1* and *mcr-2* have been found in commensal and pathogenic *Escherichia coli* isolated from food-producing animals (Callens et al., 2016; Malhotra-Kumar et al., 2016; Xavier et al., 2016; CODA-CERVA, 2017), and in *Salmonella enterica* strains from chicken meat (Nadine Botteldoorn, WIV-ISP, personal communication). Although human salmonellosis is most frequently treated with fluoroquinolones, the presence of transferable colistin resistance mechanisms in some *Salmonella* spp. might represent a risk as this resistance might be transferred to other commensal or pathogenic bacteria in humans.

A limited antimicrobial usage in laying hens has been reported (van Hoorebeke et al., 2011). Antimicrobial resistance data from indicator bacterium *E. coli* isolated from laying hens can confirm the low antimicrobial selection pressure in the laying hen sector, yet no data are available for Belgium. In broiler chickens, however, antimicrobial resistance levels in *E. coli* are yearly monitored as part of the national monitoring program in food-producing animals. In 2016, only 8.4% of the strains were fully susceptible (CODA-CERVA, 2017). It has been estimated that on average, in broiler chickens, antimicrobials are being administered during 12% of their life (Persoons et al., 2012). Highest levels of antimicrobial resistance are seen for sulfamethoxazole, ampicillin, trimethoprim, tetracycline, ciprofloxacin and nalidixic acid in both *Salmonella* spp. and *E. coli* from broiler chickens (CODA-CERVA, 2017). Even higher levels of antimicrobial resistance to sulfamethoxazole, ampicillin, trimethoprim, tetracycline are present compared to previous year (CODA-CERVA, 2016). The

abovementioned antimicrobials are frequently listed as first and second choice in the treatment of poultry-associated diseases (AMCRA, 2014). Also, these antimicrobials belong to the most used classes of antimicrobials in animals in 2014 (BelVet-SAC, 2015). Results from the national data collection system for food-producing animals, i.e. poultry, mandatory from the 27th of February 2017, will provide insight in the antimicrobial usage patterns in broiler chicken and laying hen farms. The relation between antimicrobial use and resistance will therefore be accurately investigated in the coming years.

Resistance to ciprofloxacin and nalidixic acid, both quinolones, is frequently present in *Salmonella* spp. isolated from broiler chickens (44.9% and 44.9% respectively), and almost doubled compared to previous year. It should be noted that *S. Infantis*, the serovar highly responsible for the presence of quinolone resistance, was more frequently isolated than previous year (20.1% and 40.9% in 2015 and 2016 respectively) (CODA-CERVA, 2016). Nevertheless, in 2016, more *S. Infantis* strains were found resistant to ciprofloxacin and nalidixic acid (85.2% and 92.3% in 2015 and 2016 respectively). In *E. coli* from broiler chickens, quinolone resistance is also very high (57.5% and 48.5% to ciprofloxacin and nalidixic acid respectively), but the levels of antimicrobial resistance decreased as compared to 2015 (CODA-CERVA, 2017). Development of resistance to fluoroquinolones occurs mainly by mutations, yet, plasmid mediated quinolone resistance (pMQR) has emerged. Overall, ciprofloxacin resistance coincided with nalidixic acid resistance in the tested *Salmonella* spp. strains, indicating absence of pMQR in these strains (Strahilevitz et al., 2009). In Belgium, fluoroquinolones, and more precisely enrofloxacin and flumequine, are being widely used in poultry for the treatment of several infections, e.g. colibacillosis, and mycoplasmosis (Persoons et al., 2012). From the 21st of July 2016, a new royal decree drastically restricts the use of fluoroquinolones and 3rd and 4th generation cephalosporins in food-producing animals in Belgium. Its implementation should discourage the use of fluoroquinolones in food-producing animals in Belgium and will hopefully result in a decreased selection and spread of quinolone resistant strains. In the Netherlands, antimicrobial resistance to fluoroquinolones has dramatically decreased since their restricted use (Dorado-Garcia et al., 2016). Resistance to ciprofloxacin and nalidixic acid was observed in many multi-resistance patterns, most frequently in *S. Infantis* and in combination with sulfamethoxazole, indicating clonal expansion of some particular *S. Infantis* lineages.

Other serovars, such as *S. Enteritidis*, *S. Typhimurium*, *S. Derby* and *S. Paratyphi B* variant Java, serovars possibly involved in human salmonellosis, were also found resistant to various extents. Yet, given the relative low number of these serovars, correct estimation of the quinolone resistance prevalence in these serovars is elusive. Fluoroquinolones are widely regarded as the treatment of choice for severe salmonellosis in humans. Fluoroquinolone resistance is therefore a serious threat for the successful treatment of salmonellosis. No high-level resistance (MIC > 4 mg/l) was seen in *Salmonella* spp. from poultry. Yet, the European Committee on Antimicrobial Susceptibility (EUCAST) recommends a clinical breakpoint of 0.06 mg/l as there is clinical evidence for a poor response to ciprofloxacin in systemic infections caused by *Salmonella* spp. displaying low levels of resistance (MIC > 0.06 mg/l) (EUCAST, 2016). Ciprofloxacin-resistant *Salmonella* spp. strains from poultry would therefore be difficult to treat with fluoroquinolones when infecting humans.

As for fluoroquinolones, 3rd generation cephalosporins are effective and critically important for treating human salmonellosis. In poultry, cephalosporins are not licensed, although ceftiofur has been used off-label in one-day-old chickens at the hatchery, resulting in high ceftiofur resistance in commensal *E. coli* from broiler chickens (Persoons et al., 2010). In general, ESBLs and/or AmpC are

less prevalent in *Salmonella* spp. than in *E. coli*. Yet, cephalosporin resistance has been reported to increase in *S. Paratyphi* B variant Java from broilers in Belgium, the Netherlands and Germany (Doublet et al., 2014). The specific detection of ESBL- and/or AmpC-producing *Salmonella* spp. was not in the scope of the national monitoring study on antimicrobial resistance of *Salmonella* spp. The random sampling from non-selective culture plates didn't detect any *Salmonella* spp. resistant to cefotaxime and ceftazidime in broiler chickens and laying hens. In 2015, only one *S. Typhimurium* was found cephalosporin resistant (CODA-CERVA, 2016). In *Salmonella* spp. from poultry meat cephalosporin resistance was not detected in 2015, yet 7 ESBL-producing *Salmonella* spp. out of 176 (3.97%) *Salmonella* strains were found on chicken carcasses in 2016 (Garcia-Graells, Cristina, WIV-ISP, personal communication). Different outcomes in antimicrobial resistance in bacteria from living animals and carcasses are indicative for cross-contamination with human sources or slaughterhouse equipment.

For the third year in a row, antimicrobials considered as last resort for treatment of extremely antimicrobial resistant isolates in humans, were tested for their antibacterial activity on *Salmonella* spp., i.e. azithromycin, meropenem and tigecycline.

Absence of meropenem resistance indicated that carbapenemase producers were not present in the tested *Salmonella* isolates. Carbapenems is a class of antimicrobials not used in food-producing animals, but reserved for humans. Yet, carbapenemase-producing *S. enterica* have been found in broiler chicken farms in Germany (Fisher et al., 2013; Poirel et al., 2012). In Belgium, carbapenem resistance has been reported in *Acinetobacter* spp. from horses (Smet et al., 2012) and in one commensal *E. coli* strain from pig meat (EFSA and ECDC, 2017).

Tigecycline, structurally related to tetracycline, but with a broader spectrum of activity, is used as a last resort molecule in the treatment of ESBL-infected patients in human medicine. It has no veterinary equivalent; and in contrast to previous year, clinically relevant resistance (clinical breakpoint is 2 mg/l), was not detected in *Salmonella* spp. from broiler chickens.

Supplementary data

Table 1: Minimum Inhibitory Concentrations for *Salmonella* spp. strains (n= 127), isolated from broiler chickens, using non-selective media for ampicillin (AMP), azithromycin (AZI), chloramphenicol (CHL), ciprofloxacin (CIP), colistin (COL), cefotaxime (FOT), gentamicin (GEN), meropenem (MERO), nalidixic acid (NAL), sulfomethoxazole (SMX), ceftazidime (TAZ), tetracycline (TET), tigecycline (TGC) and trimethoprim (TMP). Epidemiological cut-off's (ECOFFs) are indicated as straight lines (|).

	<=0.008	<=0.015	<=0.03	<=0.06	<=0.12	<=0.25	<=0.5	<=1	<=2	<=4	<=8	16	32	64	128	256	512	1024	2048
AMP	-	-	-	-	-	-	-	34	39	11	0	0	0	43	-	-	-	-	-
AZI	-	-	-	-	-	-	-	-	4	46	62	15	0	0	0	-	-	-	-
CHL	-	-	-	-	-	-	-	-	-	-	100	22	0	0	0	5	-	-	-
CIP	-	54	16	0	5	17	24	10	1	0	0	0	-	-	-	-	-	-	-
COL	-	-	-	-	-	-	-	125	0	2	0	-	-	-	-	-	-	-	-
FOT	-	-	-	-	-	125	2	0	0	0	0	-	-	-	-	-	-	-	-
GEN	-	-	-	-	-	-	123	0	1	0	0	2	1	0	-	-	-	-	-
MERO	-	-	123	4	0	0	0	0	0	0	0	0	-	-	-	-	-	-	-
NAL	-	-	-	-	-	-	-	-	-	70	0	0	0	0	13	44	-	-	-
SMX	-	-	-	-	-	-	-	-	-	-	2	1	15	25	2	0	0	0	82
TAZ	-	-	-	-	-	-	121	6	0	0	0	0	-	-	-	-	-	-	-
TET	-	-	-	-	-	-	-	-	75	8	0	0	0	3	41	-	-	-	-
TGC	-	-	-	-	-	69	31	27	0	0	0	-	-	-	-	-	-	-	-
TMP	-	-	-	-	-	59	29	1	0	0	0	0	0	38	-	-	-	-	-

Table 2: Minimum Inhibitory Concentrations for *Salmonella* spp. strains (n= 39), isolated from laying hens, using non-selective media for ampicillin (AMP), azithromycin (AZI), chloramphenicol (CHL), ciprofloxacin (CIP), colistin (COL), cefotaxime (FOT), gentamicin (GEN), meropenem (MERO), nalidixic acid (NAL), sulfomethoxazole (SMX), ceftazidime (TAZ), tetracycline (TET), tigecycline (TGC) and trimethoprim (TMP). Epidemiological cut-off's (ECOFFs) are indicated as straight lines (|).

	<=0.008	<=0.015	<=0.03	<=0.06	<=0.12	<=0.25	<=0.5	<=1	<=2	<=4	<=8	16	32	64	128	256	512	1024	2048
AMP	-	-	-	-	-	-	-	32	7	0	0	0	0	0	-	-	-	-	-
AZI	-	-	-	-	-	-	-	-	1	26	12	0	0	0	0	-	-	-	-
CHL	-	-	-	-	-	-	-	-	-	-	39	0	0	0	0	0	-	-	-
CIP	-	31	7	1	0	0	0	0	0	0	0	0	-	-	-	-	-	-	-
COL	-	-	-	-	-	-	-	34	5	0	0	-	-	-	-	-	-	-	-
FOT	-	-	-	-	-	39	0	0	0	0	0	-	-	-	-	-	-	-	-
GEN	-	-	-	-	-	-	38	1	0	0	0	0	0	0	-	-	-	-	-
MERO	-	-	38	1	0	0	0	0	0	0	0	0	-	-	-	-	-	-	-
NAL	-	-	-	-	-	-	-	-	-	39	0	0	0	0	0	0	-	-	-
SMX	-	-	-	-	-	-	-	-	-	-	0	0	4	26	9	0	0	0	0
TAZ	-	-	-	-	-	-	39	0	0	0	0	0	-	-	-	-	-	-	-
TET	-	-	-	-	-	-	-	-	39	0	0	0	0	0	0	-	-	-	-
TGC	-	-	-	-	-	38	1	0	0	0	0	-	-	-	-	-	-	-	-
TMP	-	-	-	-	-	33	6	0	0	0	0	0	0	0	-	-	-	-	-

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