Programmed surveillance of *Salmonella* spp. contamination of fresh poultry meat at slaughterhouse and the antimicrobial resistance of strains isolated in 2014

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Abstract

Programmed surveillance of *Salmonella* spp. contamination of fresh poultry meat at slaughterhouse and the antimicrobial resistance of strains isolated in 2014. In 2014, implementing Decision 2013/652/EU on the surveillance and reporting of antimicrobial resistance in zoonotic and commensal bacteria, the Directorate General for Food (DGAL) organised a surveillance programme on poultry carcass contamination by *Salmonella* spp. at slaughterhouse. The antimicrobial resistance of these *Salmonella* isolates was also assessed. In order to produce data representative of the slaughtered volume nationwide, only certified poultry slaughterhouses were targeted in mainland and overseas France. Contamination by *Salmonella* spp. was on average greater than 10%. Turkey carcasses displayed higher contamination rates than chicken carcasses. The most commonly observed serovars were not those regulated in fresh poultry meat. Therefore, non-compliance rates remained very low, at around 1%. The resistance profiles observed rarely involved critically important antibiotics for human health. Multi-drug resistance appeared to be quite rare in chickens, while it was more frequent in turkeys. This programme is designed to be reproduced every other year in order to provide temporal trends as well as comparable data at European level.

Keywords

Monitoring program, *Salmonella*, Poultry, Carcasses, Antimicrobial resistance

Résumé

Surveillance programmée de la contamination par* Salmonella *spp. des viandes fraîches de volaille au stade de l'abattoir et de la résistance aux antibiotiques des souches isolées en 2014

En application de la décision 2013/652/UE concernant la surveillance de la résistance aux antimicrobiens chez les bactéries zoonotiques et commensales, la direction générale de l'Alimentation a organisé en 2014 un plan de surveillance de la contamination par Salmonella *spp. des carcasses de volailles au stade de l'abattage et de la résistance aux antibiotiques des souches isolées. Seuls les abattoirs de volailles agréés dans l'ensemble des régions de France métropolitaine et d'Outre-mer étaient concernés afin de produire une information représentative des volumes d'abattage au niveau national. Le taux de contamination moyen des carcasses de volailles par* Salmonella *est supérieur à 10 %. Les carcasses de dindes présentent un taux de contamination plus élevé que celles de poulets. Les sérovars majoritairement isolés ne sont pas ceux qui sont concernés par le critère réglementaire de sécurité défini pour les viandes fraîches de volailles dans le règlement (CE) n°2073/2005 ; les taux de non-conformité sont donc faibles, proches de 1 %. Les profils d'antibiorésistance obtenus concernent peu les antibiotiques critiques pour la santé humaine. Par ailleurs, si les souches multi-résistantes sont peu nombreuses chez le poulet, leur nombre est plus élevé chez la dinde. Ce plan est destiné à être reconduit les années paires afin de comparer l'évolution du niveau de résistance des souches de* Salmonella *isolées au sein de ces filières, au niveau européen.*

Mots-clés

Plan de surveillance, Salmonella, *volaille, carcasses, antibiorésistance*

Salmonella is the second-leading cause of food-borne infections in humans and remains the most common cause of food-borne outbreaks of bacterial origin in Europe. The principal reservoir of *Salmonella* is the gastro-intestinal tract of mammals (pigs and cattle) and birds (domestic poultry). Transmission to humans mostly occurs through the consumption of raw or undercooked contaminated foods. For the most susceptible individuals, antimicrobials are administered to treat salmonellosis. However, the bacteria can acquire patterns of antimicrobial resistance and therefore resist treatments. This phenomenon is a public health threat.

In accordance with Directive 2003/99/EC, European Union Member States are required to set up a surveillance system for zoonoses, zoonotic agents and related antimicrobial resistance. *Salmonella* are included on the list of agents to be monitored featured in Annex I (A) of this directive. For food-borne *Salmonella*, official surveillance consists in: i) supervision of the implementation of Regulation (EC) No 2073/2005 by operators, and ii) implementation of Decision 2013/652/EU on the monitoring and reporting of antimicrobial resistance in zoonotic and commensal bacteria.

The main objective of this surveillance programme was to characterise the antimicrobial susceptibility profiles of the *Salmonella* strains isolated from poultry carcasses at the slaughterhouse, in accordance with Decision 2013/652/EU. The programme also provided for verification of the compliance of poultry carcasses with microbiological safety criterion 1.28 of Regulation (EC) No 2073/2005, introduced in 2011, for *Salmonella* Typhimurium (including its monophasic variant 1,4,[5],12:i:-) and *Salmonella* Enteritidis.

Box.

Objectives

Descriptive study on the antimicrobial susceptibility of *Salmonella* strains isolated from poultry carcasses at the slaughterhouse.

Verification of the compliance of poultry carcasses with the regulatory safety criterion.

Framework

Decision 2013/652/EU, Regulation (EC) No 2073/2005 (safety criterion 1.28).

Protocol

The sampling plan was designed to obtain 170 *Salmonella* isolates in the chicken sector and 170 *Salmonella* isolates in the turkey sector.

- **Nature of the tested contaminants:** *Salmonella*, susceptibility to 14 antimicrobials representing 12 antimicrobial classes.
- **Affected products ("population"):** turkey and chicken carcasses at the slaughterhouse
- **Definition of a "case":** a sample was considered non-compliant if it was contaminated by *Salmonella* Enteritidis or Typhimurium (including its monophasic variant 1,4,[5],12:i:-).

Number of samples and sampling method: 3000 (1200 samples from fattening turkeys and 1800 samples from broiler chickens) in proportion to slaughter volumes.

- **Sampling strategy:** random in each slaughterhouse.
- **Analytical method, nature of sampling:** *Salmonella* testing on neck skin according to reference method NF EN ISO 6579 "Microbiology of foods – Horizontal method for the detection of *Salmonella*" or equivalent alternative methods validated by AFNOR Certification.

Materials and methods

Sampling protocol

In accordance with Decision 2013/652/EU, the sampling plan was designed so as to obtain 170 *Salmonella* isolates in the chicken sector and 170 in the turkey sector to test their antimicrobial susceptibility.

The number of samples was calculated based on the results of a similar surveillance programme, implemented by the Directorate General for Food (DGAL) in 2010 (average contamination rate in chicken carcasses: 10.4% and in turkey carcasses: 16.7%)[1].

Thus, taking a margin of safety into account, assuming a decrease in *Salmonella* prevalence in poultry carcasses related to the introduction of microbiological safety criterion 1.28, the total number of samples was set at 3000 (1200 samples from fattening turkeys and 1800 samples from broiler chickens).

The samples were spread out across eighteen regions and three overseas territories, in proportion to the slaughter volumes of accredited poultry slaughterhouses. The samples were then divided up between the various slaughterhouses by the regions, in accordance with the protocol on the organisation of surveillance and control plans defined by the DGAL, which specifies, among other things, requirements for the geographic and temporal distribution of samples (distribution in proportion to slaughter volumes, smoothing of samples throughout the year).

Sampling and sending to laboratories

The batches to be sampled were to be randomly selected. In accordance with Regulation (EC) No 2073/2005 (Annex I, Chapter 3), the samples were made up of five units of poultry neck skin (n=5), prepared as follows (Figure 1):

- an approximately 10g piece of neck skin was collected from fifteen randomly selected poultry carcasses from the same original holding, after chilling,

1. http://agriculture.gouv.fr/sites/minagri/files/documents/pdf/recueil_tt_publicPSPC_2010_v4.pdf.

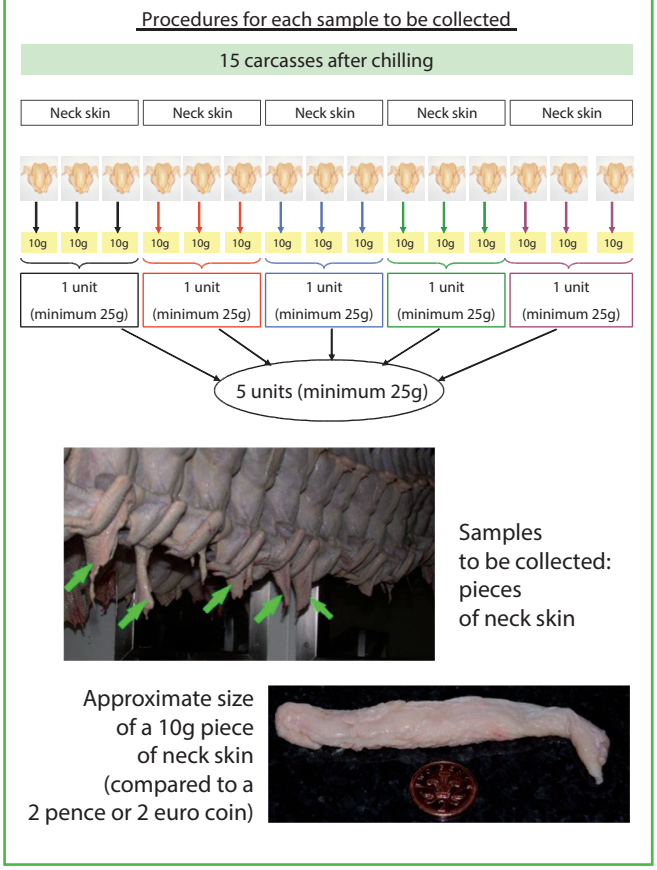

Figure 1. Sampling procedure (extracted from technical instruction DGAL/SDSSA/2013-9926 of 24/12/2013)

Table 1. List of tested antimicrobials and interpretative thresholds according to EUCAST (www.eucast.org)

Antimicrobial class	Tested antimicrobial (abbreviation)	Epidemiological cut-off values (ECOFFs) (mg/L)
Penicillins	Ampicillin (AMP)	> 8
3GC	Cefotaxime (CTX)	> 0.5
	Ceftazidime (CAZ)	> 2
Carbapenems	Meropenem (MEM)	> 0.125
Macrolides	Azithromycin (AZM)	> 16*
(Fluoro)quinolones	Nalidixic acid (NAL)	> 16
	Ciprofloxacin (CIP)	> 0.064
Aminoglycosides	Gentamicin (GEN)	> 2
Phenicols	Chloramphenicol (CHL)	> 16
Sulfonamides	Sulfamethoxazole (SSS)	> 256*
Diaminopyrimidines	Trimethoprim (TMP)	> 2
Tetracyclines	Tetracycline (TET)	> 8
Glycylcyclines	Tigecycline (TGC)	> 1
Polymyxins	Colistin (CST)	> 2

*: cut-off values not provided by EUCAST (http://www.eucast.org/mic_distributions_and_ecoffs/), values used on a proposal from the European Union Reference Laboratory (EURL)-Antimicrobial resistance (http://www.crl-ar.eu/)

- then the pieces of neck skin from three carcasses were pooled in order to form five units with the minimum weight of 25g required for the analysis.

The samples were sent to the analytical laboratories accredited for the detection and serotyping of *Salmonella*. The isolated *Salmonella* strains were then sent to ANSES in Maisons-Alfort for the analysis of their antimicrobial susceptibility.

Analytical methods

Salmonella detection and serotyping

Salmonella detection and serotyping in the isolated strains were undertaken according to reference method NF EN ISO 6579 "Microbiology of foods – Horizontal method for the detection of *Salmonella*". Equivalent alternative methods validated by AFNOR Certification were authorised if they had no restrictions for use.

Analysis of the antimicrobial susceptibility of the isolated strains

The antimicrobial susceptibility profile was determined by microdilution in a liquid medium according to the Sensititre® method. The Minimum Inhibitory Concentration (MIC) of fourteen antimicrobials, representing twelve antimicrobial classes, was measured. The interpretative thresholds used were those listed in Decision 2013/652/EU. These are the epidemiological cut-off values determined by the European Committee on Antimicrobial Susceptibility Testing (EUCAST). For non-determined (ND) values, the European Food Safety Authority (EFSA) provided temporary interpretative values (Table 1). These cut-offs may change as knowledge is improved and data are accumulated. A resistance phenotype is said to be "wild-type" when the bacterium shows no acquired resistance. Wild-type *Salmonella* are naturally susceptible to the 14 tested antimicrobials. "Multidrug resistance" is defined as the acquisition of resistance to at least three classes of antimicrobials (EFSA and ECDC, 2016).

Results

In total, 1183 samples from fattening turkeys and 1696 samples from broiler chickens were analysed, corresponding to sampling rates of 98.5% and 94% respectively for the plan. These samples were collected from a total of 131 slaughterhouses (20% of French slaughterhouses slaughtering poultry).

Contamination rates and verification of compliance with the safety criterion

Broiler chickens

Of the 1696 samples collected from broiler chickens in 122 slaughterhouses, *Salmonella* was detected in 210 samples from 26 slaughterhouses (21% of the included slaughterhouses), corresponding to an average carcass contamination rate of 12.4%.

Nineteen different serovars were identified; the most common were Derby (29%), Anatum (27%) and Indiana (20%). Serovar Typhimurium (including its monophasic variant 1,4,[5],12:i:-) was found in 10 samples and serovar Enteritidis was not isolated, corresponding to an estimated regulatory non-compliance rate of 0.6% for broiler chickens.

Fattening turkeys

Of the 1183 samples collected from fattening turkeys in 27 slaughterhouses, *Salmonella* was detected in 192 samples from 15 slaughterhouses (111 contaminated samples came from the same slaughterhouse), corresponding to an average carcass contamination rate of 16.2%. Sixteen different serovars were identified; the most common were Bredeney (41%), Anatum (14%) and Saintpaul (12%). Serovar Typhimurium (including its monophasic variant 1,4,[5],12:i:-) was found in 14 samples and serovar Enteritidis was found in one sample, corresponding to an estimated regulatory non-compliance rate of 1.3% for fattening turkeys.

Analysis of antimicrobial susceptibility

After the exclusion of isolates that were received in duplicate[2] or were contaminated[3], 169 *Salmonella* strains isolated from chicken carcasses and 173 *Salmonella* strains isolated from turkey carcasses were analysed to test their antimicrobial susceptibility.

Broiler chickens

In total, 154 wild-type strains (91.1%) were observed, 11 strains (6.5%) had a phenotype of resistance to one or two antimicrobial classes, and four strains (2.4%) were multi-drug resistant (Figure 2). Production of extended-spectrum beta-lactamases (ESBLs) was not observed. No resistance to third-generation cephalosporins (3GC) or carbapenems was detected. Resistance to ciprofloxacin accounted for 1.2% of strains. Resistance to colistin accounted for 2.4% (Table 2).

All the isolates of the main serovar, Derby, were wild-type. For Typhimurium, resistance was observed for ampicillin, sulfonamides and tetracycline, while the monophasic variants of Typhimurium were resistant to ampicillin and sulfonamides.

Fattening turkeys

In total, 54 wild-type isolates (31.2%) were observed, 79 strains (45.7%) had a phenotype of resistance to one or two antimicrobial classes, and 40 strains (23.1%) were multidrug resistant (Figure 2).

As in the chicken sector, no ESBL production and no resistance to 3GC or carbapenems were observed. Resistance to ciprofloxacin accounted for 6.9% of strains; as for colistin, 38.7% of strains had a MIC value just above the ECOFF.

Strains of the main serovar, Bredeney, displayed a variety of resistance profiles. It should be noted that a high percentage of strains were resistant to tetracycline (53/56) and/or had a MIC for colistin (37/56) slightly above the cut-off value.

For regulated serovars in the framework of the *Salmonella* controle and eradication l European programme in the poultry sector, the two *S.* Hadar strains were resistant to nalidixic acid and tetracycline. Strains of *S.* Typhimurium and its monophasic variant showed homogeneous resistance: they were all resistant to ampicillin, sulfonamides, tetracycline and gentamicin. However, one *S.* Typhimurium strain and one monophasic variant also had a MIC for colistin above the epidemiological cut-off value, classifying them as resistant to this antimicrobial.

The distribution of "resistance" to colistin was highly heterogeneous between serovars (majority of *S.* Bredeney and *S.* Brandenburg, some *S.* Anatum, *S.* Albany, *S.* Newport, *S.* Indiana, *S.* Montevideo, *S.* Eko). Most of the colistin-resistant strains had a MIC of 4 mg/L, i.e. the value just above the cut-off, which is not a result indicative of true resistance. Moreover, an antibiogram on agar medium did not provide confirmation of the colistin resistance of these *Salmonella*. However, a serovar Brandenburg strain had a MIC for colistin of 8 mg/L. For this strain, an antibiogram on agar medium showed an inhibition zone diameter of 9 mm around the 10 µg colistin disk. This is significantly narrower than what is typically obtained with *Salmonella* (approximately 15 mm) and suggests a colistin-resistance mechanism. Testing for the *mcr-1* gene, the sole mechanism of plasmid-mediated colistin resistance described before the summer of 2016 (Box), did not evidence this type of mechanism for this strain.

Discussion - conclusion

The average *Salmonella* contamination rate in poultry carcasses at the slaughterhouse was approximately 10% and appeared higher in the fattening turkey sector than in the broiler chicken sector. In this respect, the results obtained in 2014 were similar to the results of the 2010 surveillance programme obtained from fewer samples. Nonetheless, these results should be interpreted with caution, given the variability observed between slaughterhouses. Contamination rates in poultry carcasses

depended on several factors, such as slaughter volumes and rates, processes, farm contamination levels, etc. More in-depth studies into these risk factors would provide confirmation of these assumptions. Furthermore, several selection biases may have caused the results

2. All isolates from the same sample with the same serovar were considered as duplicates. In this case, only one copy was kept, which became a strain.
3. The stage of *Salmonella* detection in the sample must have been followed by a purification stage before sending to the NRL for Antimicrobial resistance for analysis of the resistance phenotype. Some cultures were found to be polymicrobial and could not be used.

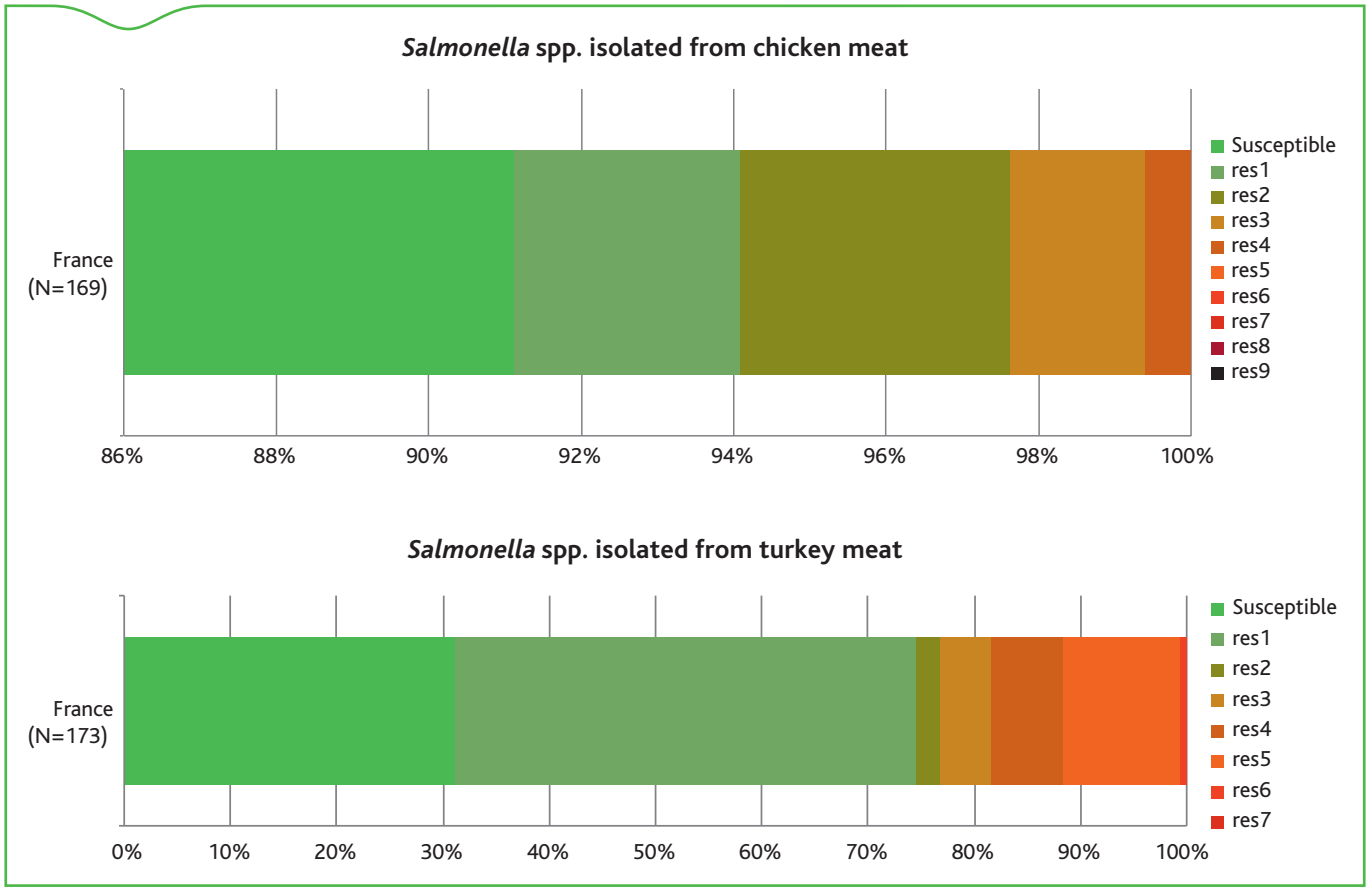

Figure 2. Distribution of resistance frequencies for *Salmonella* strains isolated from turkey and chicken carcasses in 2014 in France, expressed by antimicrobial class (according to EFSA and ECDC, 2016). Multi-drug resistance is defined as the acquisition of resistance to at least three classes of antimicrobials

to be overestimated, in particular possible non-compliance with the sampling strategy by some samplers preferring to sample batches from farms found positive for *Salmonella*.

It should be noted that control measures in the poultry sector seem to have limited the presence of the five serovars covered by eradication programmes (*S.* Typhimurium and its monophasic variant, *S.* Enteritidis, *S.* Hadar, *S.* Infantis, *S.* Virchow). These serovars were not those mainly found in poultry carcasses. This underlines the importance of taking all *Salmonella* serovars into account in the health control plans of operators downstream of slaughterhouses.

According to the results of the 2014 and 2010 surveillance programmes, the introduction of safety criterion 1.28 in Regulation (EC) No 2073/2005 in 2011 had no impact on *Salmonella* contamination rates in poultry carcasses. The rate of regulatory non-compliance for poultry carcasses (presence of serovar Typhimurium (including its monophasic variant 1,4,[5],12:i:-) or Enteritidis) was close to 1%. The management of non-compliant batches led to the withdrawal of their carcasses and cuts of meat, in accordance with the guide to the management of food alerts(4)[4].

As for the analysis of antimicrobial susceptibility, most of the *Salmonella* strains isolated in the chicken sector were wild-type. Rates of resistance and multi-drug resistance for

Salmonella strains were higher in the turkey sector. This finding appeared valid for all the Member States that reported data to EFSA for turkey and chicken meat (EFSA & ECDC, 2016).

It is reassuring to note that no ESBL phenotypes, 3GC resistance or carbapenem resistance were observed in *Salmonella* from the

4. http://agriculture.gouv.fr/sites/minagri/files/documents/pdf/_Guide_Gestion_Alerte_Revision_2_jlt_2009_COMPLETEE_VDef__cle09fc34.pdf.

Table 2. Rates of resistance for the isolated *Salmonella* strains by antimicrobial

Antimicrobial (Epidemiological cut-off value in mg/L)	Resistance rate (%, [95CI])	
	Broiler chickens N=169	Fattening turkeys N=173
Ampicillin (8) AMP	5.9 [3.2-10.5]	24.3 [18.5-31.2]
Cefotaxime (0.5) CTX	0.0 [0.0-2.2]	0.0 [0.0-2.2]
Ceftazidime (2) CAZ	0.0 [0.0-2.2]	0.0 [0.0-2.2]
Meropenem (0.125) MEM	0.0 [0.0-2.2]	0.0 [0.0-2.2]
Azithromycin (16) AZM	1.2 [0.3-4.2]	0.0 [0.0-2.2]
Nalidixic acid (16) NAL	0.0 [0.0-2.2]	6.4 [3.6-11.0]
Ciprofloxacin (0.06) CIP	1.2 [0.3-4.2]	6.9 [4.0-11.7]
Gentamicin (2) GEN	0.0 [0.0-2.2]	0.6 [0.1-3.2]
Chloramphenicol (16) CHL	0.6 [0.1-3.3]	10.4 [6.7-15.8]
Sulfamethoxazole (256) SSS	4.7 [2.4-9.1]	22.5 [17.0-29.3]
Trimethoprim (2) TMP	1.8 [0.6-5.1]	17.3 [12.4-23.7]
Tetracycline (8) TET	3.6 [1.6-7.5]	65.9 [58.6-72.5]
Tigecycline (1) TGC	0.0 [0.0-2.2]	1.7 [0.6-5.0]
Colistin (2) CST	2.4 [0.9-5.9]	38.7 [31.8-46.2]

chicken and turkey sectors at the slaughterhouse. There was also a low level of fluoroquinolone resistance; it was higher in the turkey sector (6.9%) but below the average rate for the other Member States (24.3%) (EFSA & ECDC, 2016). Comparison with EFSA's data is however limited to countries that reported results in these sectors and should also be considered in relation to the number of analysed strains. For example, out of 28 Member States, data from only nine Member States reporting a total of 726 analysed *Salmonella* strains were available for the entire turkey sector (farming environment and/or meat). For turkey meat specifically, only three countries (France, Germany and Hungary) reported data for 226 analysed strains. The observed colistin-resistance rate was apparently high, especially in the turkey sector. However, it should be analysed with caution due to the limitations of the method and the lack of perspective for these data. The MIC values measured by the micro-dilution method are accurate ata factor of 8, which means that 2 values measured can not be considered as different if they do not differ from a factor of 8 There were many measurements just above the cut-off value. Application of this factor of 8 did not show that these strains were definitively resistant to colistin. The collection of MIC data for colistin in future surveillance programmes, as well as further research into the topic, should shed light on these results and provide a clearer idea as to the risk of this resistance spreading. Lastly, the relevance of the cut-off value for colistin (> 2 mg/L) currently used for the "resistant" or "susceptible" interpretation should be reviewed as MIC data are accumulated.

This programme is designed to be reproduced every other year at European level. The experience acquired by the various EU Member States should facilitate the analysis and transmission of data in order to highlight any trends by country or even the circulation of antimicrobial-resistant strains.

Box. Colistin resistance

In November 2015, Liu *et al.* (2016) published the first mechanism of plasmid-mediated colistin resistance. Before that, colistin resistance had been considered as not horizontally transmissible between bacteria. The discovery of this *mcr-1* gene in China was quickly followed by descriptions of this gene all over the world, in various Enterobacteriaceae (*E. coli*, *Salmonella*, *Klebsiella pneumoniae*, etc.) and from various origins (humans, animals, food, etc.). Colistin resistance has been monitored in Europe only since the implementation of Decision 2013/652/EU on 1 January 2014. It should be noted that methods for the phenotypic characterisation of colistin resistance are still not very robust and are difficult to interpret.

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