Annex A - Materials and methods

Annex to:

EFSA (European Food Safety Authority) and ECDC (European Centre for Disease Prevention and Control), 2021. **The European Union Summary Report on Antimicrobial Resistance in zoonotic and indicator bacteria from humans, animals and food in 2018/2019.** EFSA Journal 2021;19(4):6490, doi:10.2903/j.efsa.2021.6490

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1. Antimicrobial susceptibility data from humans available in 2019

1.1. Data reported to The European Surveillance System (TESSy)

MSs report results from antimicrobial susceptibility testing of *Salmonella* spp. and *Campylobacter* spp. isolated from clinical cases to ECDC on an annual basis. Data can be submitted to ECDC and The European Surveillance System (TESSy) either as measured values (inhibition zone diameters or minimum inhibitory concentrations (MIC)) through the isolate-based reporting in TESSy or as results interpreted with clinical breakpoints via the case-based reporting of *Salmonella* and *Campylobacter* infections. New from 2019 is that data can also be submitted as phenotypes predicted from sequencing of the bacterial genome, also via the isolate-based reporting. The reporting of quantitative data via the isolate-based reporting is the preferred route, as stipulated in the EU protocol for harmonised monitoring of AMR in human *Salmonella* and *Campylobacter* isolates (ECDC, 2016).

Salmonella spp.: For 2019, 24 MSs, plus Iceland and Norway provided data on antimicrobial resistance (AMR) in human *Salmonella* isolates. Seventeen countries reported measured values and seven reported results interpreted as susceptible standard dosing regimen, susceptible increased exposure or resistant (SIR) according to the clinical breakpoints (CBPs) applied. Two countries reported results categorised as predicted wild type or predicted non-wild type based on analysis of bacterial genomes (Table 1:).

Campylobacter spp.: 19 MSs, plus Iceland and Norway provided AMR data from human isolates for 2018. Thirteen countries reported measured values and eight reported results interpreted as susceptible standard dosing regimen, susceptible increased exposure or resistant (SIR) according to the clinical breakpoints (CBPs) applied (Table 2:).

1.2. Harmonised testing

Most laboratories follow the 'EU protocol for harmonised monitoring of antimicrobial resistance in human *Salmonella* and *Campylobacter* isolates' (ECDC, 2016) on the antimicrobial panel to be tested. The antimicrobials tested, the method used (dilution, disk diffusion, gradient strip), the type of data provided and the interpretive criteria applied are presented in Table 1 for *Salmonella* and in Table 2 for *Campylobacter*. For *Salmonella*, seven MSs, plus Iceland and Norway used only disk diffusion methods (DDs) for their AST, eight MSs used dilution methods (DLs) and another six MSs used a combination of the two, mostly disk diffusion and gradient strip, depending on the situation and the antimicrobial. Two countries used sequencing and bioinformatics tools to predict phenotypic resistance from the genome. For one MS, the method of testing was not provided (Table 1:). For *Campylobacter*, nine MSs used only disk diffusion methods (DDs) for their AST, five MSs and Norway used dilution methods (DLs), three MSs and Iceland used a combination of the two, mostly disk diffusion and gradient strip, and for two MSs the methodology was not provided (Table 2:). All data on measured MIC or zone mm values were results of AST at the national public health reference laboratories, with the exception of Italy for *Salmonella* where two regional laboratories also contributed, and Finland for *Campylobacter* where the quantitative data had been collected from regional laboratories. Data interpreted with clinical breakpoints were normally from local or regional laboratories and reported together with the information on the clinical case. In these cases, AST had primarily been performed with the purpose of treatment of the case rather than AMR monitoring. For this reason, the number of tests per antimicrobial varied.

Salmonella test panel

In 2013, the national public health laboratories within the Food- and Waterborne Diseases and Zoonoses (FWD) network agreed on a panel of priority antimicrobials and optional antimicrobials to test for and report to ECDC (ECDC, 2016). Two antimicrobials – ceftazidime and meropenem – were new in the priority panel compared with earlier recommendations. For 2019, all but one MS and Iceland reported results on meropenem and all but four plus Iceland for ceftazidime. It was also agreed that three last-line antimicrobials – azithromycin, colistin and tigecycline – should be included in the priority list. For colistin, however, the methodology is complicated due to chemical properties of the substance and a joint EUCAST and Clinical and Laboratory Standards Institute (CLSI) subcommittee confirmed that broth microdilution is so far the only valid method for colistin susceptibility testing (CLSI and EUCAST, 2016). Disk diffusion does not work because of poor diffusion of the large colistin molecule in the agar and tested gradient strips also underestimate colistin MIC values, again most likely due to poor diffusion in the agar (Matuschek et al., 2017). The three last-line antimicrobials were added to the priority list in

June 2016 (ECDC, 2016), however only countries performing broth microdilution should report on colistin resistance. Eight MSs were reporting on azithromycin, eight on tigecycline and seven on colistin for 2019.

Due to the problems in detecting low-level fluoroquinolone resistance in *Salmonella* spp. using disk diffusion, nalidixic acid was, for a long time, used as a marker for fluoroquinolone resistance. After the discovery that plasmid-mediated fluoroquinolone resistance is often not detected using nalidixic acid, EUCAST studied alternative disks and concluded that pefloxacin was an excellent surrogate marker (except for isolates having the *aac*(*6'*)-*Ib-cr* gene as the only resistance determinant) (Skov et al., 2015). Since 2014, EUCAST has recommend this agent for screening of low-level fluoroquinolone resistance in *Salmonella* with disk diffusion (EUCAST, 2014) and, since June 2016, this is also reflected in the EU protocol. In 2019, all countries reporting measured values for disk diffusion tested with pefloxacin instead of ciprofloxacin. Eleven countries reported the combination drug co-trimoxazole (trimethoprim–sulfamethoxazole) in addition to, or instead of, testing the substances separately, partly because this combination is used for clinical treatment and partly because no EUCAST interpretive criterion exists for sulfamethoxazole for *Salmonella*.

Campylobacter test panel

The antimicrobials included in the 2019 report followed the panel of antimicrobials from the EU protocol for harmonised monitoring of AMR in human *Salmonella* and *Campylobacter* isolates (ECDC, 2016). The priority panel for *Campylobacter* includes ciprofloxacin, erythromycin, tetracyclines and, since June 2016, gentamicin. Gentamicin is recommended for screening of invasive isolates and was added to the priority panel after a EUCAST ECOFF became available for disk diffusion for *C. jejuni*. Co-amoxiclav (combination drug with amoxicillin and clavulanic acid) was included from the list of optional antimicrobials. In 2019, all countries except Iceland tested the three antimicrobials ciprofloxacin, erythromycin and tetracycline, 14 also tested gentamicin and seven tested co-amoxiclav.

1.3. Analyses of antimicrobial resistance data

1.3.1. Harmonised interpretation of data with animal and food data

Data reported as measured values were interpreted by ECDC based on the EUCAST ECOFF values, when available. For MIC data, the same criteria were applied as used by EFSA (Table 5: and Table 6:) while for zone diameter data, corresponding EUCAST disk diffusion ECOFF values were applied with a few exceptions (Table 1: and Table 2:). Regarding data reported as SIR values, the categories of 'susceptible, increased exposure' (I) and 'clinically' resistant (R) were combined into one group, except for tetracycline. Alignment of the susceptible category with the 'wild type' category based on epidemiological cut-off values (ECOFFs) and of the I+R category with the ECOFF-based 'non-wild type' category provides better comparability and more straightforward interpretation of the data for most antimicrobial agents included (Figure 27 in Section 3.4 and Figure D.1 in Appendix D of the 2019 EUSR-AMR). For *Salmonella*, this procedure results in good concordance (± 1 dilution) across categories with the exception of meropenem where the MIC for non-susceptible category is substantially higher (+ 4 dilutions) than the ECOFF. For *Campylobacter*, there was total concordance across interpretive categories with this procedure, except for the EUCAST CBP for *C. jejuni* for tetracyclines, which is one dilution step higher than the EUCAST ECOFF.

1.3.2. Separation by species or serovar

As resistance levels differ substantially between *Salmonella* serovars, results are presented separately for selected serovars of importance in humans. The serovars presented in the report are *S.* Enteritidis, *S.* Typhimurium, monophasic *S.* Typhimurium, *S.* Infantis, *S.* Derby and *S.* Kentucky. AMR data on the 10 most common serovars in human cases in the last years are also available in the ECDC Surveillance Atlas for Infectious Diseases (https://atlas.ecdc.europa.eu/public/index.aspx). For *Campylobacter*, resistance levels differ quite substantially between the two most important *Campylobacter* species, *C. jejuni* and *C. coli*, and data are therefore presented by species. The proportion of resistant isolates is only shown when at least 10 isolates were reported from a MS.

1.3.3. Exclusion of travel-associated cases

To better assess the impact from food consumed within each reporting country on the AMR levels found in human isolates, cases known to have travelled outside of the country during the incubation period was excluded from the analysis. However, as several countries had not provided any information on travel status of their cases, cases with unknown travel status were also included in addition to domestically-acquired cases. The proportions of travel-associated, domestic and unknown cases among the tested isolates are presented in Table 3: and Table 4: .

1.3.4. Temporal trends in resistance

Trends in the proportion of resistant isolates to selected antimicrobials over the five-year period 2015-2019 were analysed by country. The statistical significance was assessed with logistic regression in Stata 16.0 and a *p*-value of <0.05 was considered to be significant. Only countries testing at least ten isolates per year and for at least 3 years in the 5-year period were included. For *Salmonella*, the antimicrobials analysed were ciprofloxacin/pefloxacin/nalidixic acid, cefotaxime, ampicillin and tetracycline. For *Campylobacter*, the corresponding antimicrobials were ciprofloxacin, erythromycin and tetracycline.

1.3.5. Maps for critically important antimicrobials resistance

For *Salmonella*, the proportions of human isolates resistant to both of the critically important antimicrobials for treatment of severe *Salmonella* infections (WHO, 2019), fluoroquinolones (ciprofloxacin/pefloxacin) and cephalosporins (cefotaxime), were presented in maps to provide an overview of the geographical distribution of resistance in the EU/EEA. Combined 'microbiological resistance' was presented for *Salmonella* spp and the selected serovars, some included in the report and some only in the Excel appendix files). In addition, a map of ciprofloxacin/pefloxacin resistance in *S*. Kentucky isolated from humans was included in Appendix A - high-level resistance to ciprofloxacin. For *Campylobacter*, the proportions of human isolates resistant to both of the critically important antimicrobials for treatment of severe *Campylobacter* infections (WHO, 2019), fluoroquinolones (ciprofloxacin) and macrolides (erythromycin), were presented in maps to provide an overview of the geographical distribution of resistance in the EU/EEA. Combined 'microbiological' resistance (using EUCAST ECOFFs) were presented for *C. jejuni* and *C. coli*.

1.3.6. Analysis of multidrug resistance

Multidrug resistance (MDR) of human *Salmonella* spp. to nine antimicrobial classes was analysed, these classes being harmonised between ECDC and EFSA for better comparison between the two sectors. Multidrug resistance of an isolate was defined as resistance or non-susceptibility to at least three different antimicrobial classes (Magiorakos et al., 2012). The antimicrobials included were ampicillin, cefotaxime/ceftazidime, chloramphenicol, ciprofloxacin/pefloxacin/nalidixic acid, gentamicin, meropenem, sulfonamides/sulfamethoxazole, tetracyclines and trimethoprim/trimethoprim-sulfamethoxazole (co-trimoxazole). Resistance to nalidixic acid, ciprofloxacin and pefloxacin were addressed together, as they belong to the same class of antimicrobials: quinolones. Isolates that were non-wild type or I+R to any of these antimicrobials were classified as microbiologically resistant to the class of quinolones. The same method was applied to the two third-generation cephalosporins cefotaxime and ceftazidime. Trimethoprim and co-trimoxazole were also addressed together, as a few countries had only tested for susceptibility to the combination. This approach was considered appropriate because among the countries that provided data on both trimethoprim alone and the combination co-trimoxazole, the proportion of resistant or non-susceptibles corresponded closely between the two. Multidrug resistance of a *C. jejuni* or *C. coli* isolate was defined as resistance or non-susceptibility to at least three different antimicrobial classes (Magiorakos et al., 2012). The antimicrobials in the MDR analysis were harmonised between EFSA and ECDC and included ciprofloxacin, erythromycin, gentamicin and tetracyclines.

1.3.7. Analysis of ESBL, AmpC and carbapenemase-production in *Salmonella*

All countries reported results from AST of 3rd generation cephalosporins in 2019. Those which reported findings of ESBL and/or AmpC or non-wild type results to 3rd generation cephalosporins and ampicillin, were contacted by mail to provide further details on phenotypic and/or genotypic results. Of the 15 MSs and one non-MS reporting such isolates, all except four could provide further information.

Table 1: Antimicrobials reported, methods used, type of data reported and interpretive criteria applied by MSs for human *Salmonella* AST data in 2019

Country	Gentamicin	Chloramphenicol	Ampicillin	Cefotaxime	Ceftazidime	Meropenem	Tigecycline	Nalidixic acid	Ciprofloxacin/ pefloxacin	Azithromycin	Colistin	Sulfonamides	Trimethoprim	Trimethoprim-sulfa	Tetracyclines	Method used	Quantitative (Q) or categorical (SIR or PWT/PNWT)	Interpretive criteria
Austria	•	•	•	•	•	•	•	•	•(a)	•		•	•		•	DD	Q	Interpreted by ECDC. EUCAST ECOFFs for all except CLSI CBP for SUL
Belgium	•	•	•	•	•	•	•		•	•		•	•		•	DL	Q	Interpreted by ECDC, as for Austria. EFSA criteria for AZM MIC
Cyprus	•		•	•	•	•			•		•			•		DL/DLG	Q	Interpreted by ECDC, as for Austria, except for CTX and MEM where EUCAST CBP were used.
Denmark	•	•	•	•	•	•	•	•	•	•	•	•	•		•	DL	Q	Interpreted by ECDC, as for Austria. EFSA criteria for AZM MIC
Estonia	•	•	•	•	•	•	•	•	•		•	•	•		•	DL	Q	Interpreted by ECDC, as for Austria.
Finland	•	•	•	•		•		•	•(a)				•		•	DD	Q	Interpreted by ECDC, as for Austria.
France	•	•	•	•	•	•	•	•	•	•	•	•	•		•	DL	Q	Interpreted by ECDC, as for Austria. EFSA criteria for AZM MIC
Germany	•	•	•	•	•	•		•	•(b)					•	•	DL	SIR	Breakpoints as specified in GERMAP 2015. Only R included for GEN to align with ECOFF.
Greece	•	•	•	•	•	•		•	•(a)			•	•		•	DD	Q	Interpreted by ECDC, as for Austria.
Iceland			•	•					•(a)				•	•		DD	SIR	EUCAST CBP
Ireland	•	•	•	•		•			•	•	•	•	•		•	WGS	PWT/PNWT	Sequencing results interpreted by the laboratory with BioNumerics tools for acquired AMR genes and point mutations
Italy	•	•	•	•	•	•	•	•	•(a)	•	•	•	•		•	DL/DD	Q	Interpreted by ECDC, as for Austria.
Latvia	•	•	•	•	•	•			•				•	•		DD	SIR	No recent information on guideline used.
Lithuania	•	•	•	•	•	•			•(a)				•	•	•	DL/DD	SIR	EUCAST CBP
Luxembourg	•	•	•	•	•	•			•(a)			•		•	•	DD/DLG	Q	Interpreted by ECDC, as for Austria.

Table 1: continued

Country	Gentamicin	Chloramphenicol	Ampicillin	Cefotaxime	Ceftazidime	Meropenem	Tigecycline	Nalidixic acid	Ciprofloxacin/ pefloxacin	Azithromycin	Colistin	Sulfonamides	Trimethoprim	Trimethoprim-sulfa	Tetracyclines	Method used	Quantitative (Q) or categorical (SIR or PWT/PNWT)	Interpretive criteria
Malta	•		•	•	•	•			•(b)							DL/DLG	Q	Interpreted by ECDC, as for Austria. Exception CIP where Enterobacterales CBP had to be applied due to too narrow test range.
Netherlands	•	•	•	•	•	•	•	•	•	•	•	•	•		•	DL	Q	Interpreted by ECDC, as for Austria. EFSA criteria for AZM MIC
Norway		•	•	•	•	•			•(a)						•	DD	Q	Interpreted by ECDC, as for Austria.
Poland	•	•	•	•				•	•			•		•	•		SIR	No information provided..
Portugal	•	•	•	•	•	•	•	•	•(a)	•		•	•		•	DD	Q	Interpreted by ECDC, as for Austria.
Romania	•	•	•	•	•	•		•	•(a)			•	•	•	•	DD	Q	Interpreted by ECDC, as for Austria.
Slovakia		•	•	•	•	•			•					•	•	DD/DL	SIR	No update provided. Earlier EUCAST CBP except CLSI CBP for NAL, SUL and TET.
Slovenia	•	•	•	•	•	•			•(a)			•	•	•	•	DD/DLG	Q	Interpreted by ECDC, as for Austria.
Spain	•	•	•	•	•	•		•	•(a)			•	•		•	DD	Q	Interpreted by ECDC, as for Austria.
Sweden			•	•		•			•						•	WGS	PWT/PNWT	Sequencing results interpreted by the laboratory with NCBI AMRFinderPlus and CGE ResFinder and PointFinder
United Kingdom	•	•	•	•	•	•		•	•			•	•	•	•	DD/DL/ DLG	SIR	Clinical breakpoints used varies depending on clinical microbiology laboratory

AST: antimicrobial susceptibility testing; CBP: clinical breakpoint; DD: disk diffusion; DL: dilution; DLG: dilution with gradient strip; WGS: whole genome sequencing; Q: quantitative data; SIR: susceptible standard dosing regimen, susceptible increased exposure, resistant (categorical data); PWT/PNWT: predicted wild type/predicted non-wild type (categorical); ECDC: European Centre for Disease Prevention and Control; ECOFF: epidemiological cut-off; CLSI: Clinical and Laboratory Standards Institute; EUCAST: European Committee on Antimicrobial Susceptibility Testing; NCBI: National Center for Biotechnology Information, US; CGE: Center for Genomic Epidemiology, Denmark; MIC: minimum inhibitory concentration.
AZM: azithromycin; CTX: cefotaxime; GEN: gentamicin; MEM: meropenem; NAL: nalidixic acid; SUL: sulfonamides; TET: tetracycline.

(a): Pefloxacin used in disk diffusion

(b): EUCAST Enterobacterales CBP applied which is two dilutions higher than the CBP for *Salmonella*.

Table 2: Antimicrobials reported, method used, type of data reported and interpretive criteria applied by MSs for human *Campylobacter* AST data in 2019

Country	Gentamicin	Co-amoxiclav	Ciprofloxacin	Erythromycin	Tetracyclines	Method used	Quantitative (Q) or categorical (SIR)	Interpretive criteria
Austria	•		•	•	•	DL	Q	Interpreted by ECDC. EUCAST ECOFF (CIP, ERY, GEN, TET), CA-SFM CBP 2019 (AMC)
Bulgaria			•	•	•	DD	SIR	No information provided.
Cyprus			•	•	•	DD	Q	Interpreted by ECDC, as for Austria.
Denmark	•		•	•	•	DL	Q	Interpreted by ECDC, as for Austria.
Estonia	•		•	•	•	DL	Q	Interpreted by ECDC, as for Austria.
Finland			•	•	•	DD/DLG	Q	Interpreted by ECDC, as for Austria.
France	•	•	•	•	•	DD	SIR	EUCAST CBP (CIP, ERY, TET), CA-SFM CBP (AMC, GEN)
Iceland			•	•		DD/DLG	SIR	EUCAST CBP
Italy	•		•	•	•	DD	Q	Interpreted by ECDC, as for Austria.
Lithuania			•	•	•	DD	SIR	EUCAST CBP
Luxembourg	•	•	•	•	•	DD	Q	Interpreted by ECDC, as for Austria.
Malta	•	•	•	•	•	DLG/DL	Q	Interpreted by ECDC, as for Austria.
Netherlands			•	•	•	DD/DL	SIR	EUCAST CBP
Norway	•		•	•	•	DLG	Q	Interpreted by ECDC, as for Austria.
Poland	•	•	•	•	•	No information provided	SIR	No information provided.
Portugal	•		•	•	•	DD	Q	Interpreted by ECDC, as for Austria.
Romania	•	•	•	•	•	DD	Q	Interpreted by ECDC, as for Austria.
Slovakia		•	•	•	•	No update provided	SIR	In 2013, CLSI CBP. No update since.
Slovenia			•	•	•	DD	Q	Interpreted by ECDC, as for Austria.
Spain	•	•	•	•	•	DLG	Q	Interpreted by ECDC, as for Austria.
United Kingdom			•	•	•	DD/DL/DLG	SIR	EUCAST CBP

AST: antimicrobial susceptibility testing; CA-SFM: French Society for Microbiology; CBP: clinical breakpoint; DD: disk diffusion; DL: dilution; DLG: dilution with gradient strip; ECDC: European Centre for Disease Prevention and Control; ECOFF: epidemiological cut-off; EUCAST: European Committee on Antimicrobial Susceptibility Testing; Q: quantitative data; SIR: susceptible standard dosing regimen, susceptible increased exposure, resistant (categorical data).
AMC: amoxicillin/clavulanate; CIP: ciprofloxacin; ERY: erythromycin; GEN: gentamicin; TET: tetracycline.

Table 3: Proportion of tested *Salmonella* spp. isolates from human cases associated with travel, domestic cases and cases with unknown travel information by country in 2019

Country	Total *Salmonella* tested	Travel-associated	Domestic	Unknown
	N	%	%	%
Austria	1,884	0.0	0.0	100.0
Belgium	1,083	9.0	8.2	82.8
Cyprus	96	0.0	0.0	100.0
Denmark	453	56.5	43.5	0.0
Estonia	171	10.5	62.6	26.9
Finland	115	7.0	93.0	0.0
France	1,007	16.3	12.2	71.5
Germany	4,828	2.8	97.2	0.0
Greece	50	0.0	0.0	100.0
Ireland	316	42.7	31.3	25.9
Italy	569	0.0	0.0	100.0
Latvia	89	5.6	94.4	0.0
Lithuania	731	2.1	93.6	4.4
Luxembourg	131	0.0	0.0	100.0
Malta	113	0.0	0.0	100.0
Netherlands	867	16.1	0.0	83.9
Poland	1,291	0.9	99.0	0.1
Portugal	465	0.4	99.6	0.0
Romania	147	0.0	100.0	0.0
Slovakia	709	1.0	99.0	0.0
Slovenia	386	7.3	29.5	63.2
Spain	1,418	0.3	78.0	21.7
Sweden	710	14.8	84.5	0.7
United Kingdom	5,195	27.8	19.0	53.2
Total (MSs 24)	**23,244**	**11.8**	**50.6**	**37.6**
Iceland	49	44.9	22.4	32.7
Norway	371	41.8	43.9	14.3

MSs: Member States; N: number of isolates tested.

Table 4: Proportion of tested *Campylobacter jejuni* and *C. coli* isolates from human cases associated with travel, domestic cases and cases with unknown travel information by country in 2019

Country	*C. jejuni* & *C. coli*	Travel-associated	Domestic	Unknown
	N	%	%	%
Austria	499	7.6	90.2	2.2
Bulgaria	30	3.3	0.0	96.7
Cyprus	38	0.0	2.6	97.4
Denmark	244	35.2	64.8	0.0
Estonia	327	4.3	95.7	0.0
Finland	3,415	0.0	0.0	100.0
France	7,587	0.0	0.0	100.0
Italy	107	4.7	17.8	77.6
Lithuania	830	1.2	81.6	17.2
Luxembourg	271	0.0	0.0	100.0
Malta	238	0.0	0.0	100.0
Netherlands	1,555	0.0	0.0	100.0
Poland	107	0.9	98.1	0.9
Portugal	354	0.0	100.0	0.0
Romania	17	0.0	100.0	0.0
Slovakia	1,380	0.9	99.1	0.0
Slovenia	1,084	4.5	24.5	70.9
Spain	263	0.0	94.3	5.7
United Kingdom	8,899	0.8	14.6	84.6
Total (MSs 19)	**27,873**	**2.2**	**19.8**	**78.1**
Iceland	131	35.9	28.2	35.9
Norway	497	54.1	40.2	5.6

MSs: Member States; N: number of isolates tested.

2. Antimicrobial susceptibility data from animals and food in 2018-2019

2.1. Data reported under Directive 2003/99/EC and Commission Implementing Decision 2013/652/EU

For 2019 MSs reported mandatory data collected from AMR routine monitoring in *Salmonella* spp. and indicator commensal *E. coli*, as well as from the *E. coli* specific extended spectrum β-lactamase (ESBL)-/AmpC-/carbapenemase-producing monitoring, according to Commission Implementing Decision 2013/652/EU[1].

For the routine monitoring of AMR in *Salmonella* spp., in 2019, 26 MSs and 1 non-MS reported data on meat from pigs (carcases) and 7 MSs on meat from bovine animals (carcases), 8 MSs reported data on fattening pigs, 3 MSs in calves under 1 year of age and in 2018, 19 MS and 2 non MS-reported data on meat from broilers and 9 MSs on meat from fattening turkeys, 24 MSs and 1 non-MS reported data on laying hen flocks, 25 MSs and 1 non-MSs on broiler flocks and 16 MSs on fattening turkey flocks. For the routine monitoring of AMR in indicator commensal *E. coli*, in 2019, 28 MSs and 4 non-MSs reported data on fattening pigs and 9 MSs and 3 non-MS reported on calves under 1 year, whereas in 2018 28 MSs and 4 non-MSs reported data on broilers and 11 MSs and 1 non-MS reported on fattening turkeys.

In 2018, for the routine monitoring of AMR in *Campylobacter jejuni*, 25 MSs and 4 non-MSs reported data on broilers and 10 MSs and 1 non-MS on fattening turkeys. Some data on *Campylobacter coli* was also reported on a voluntary basis.

For the specific monitoring of ESBL-/AmpC-/carbapenemase-producing *E. coli*, in 2019, all MSs and 3 non-MSs, reported data on fresh meat from pigs and bovines gathered at retail, and fattening pigs, whereas 9 MSs and 2 non-MS reported data on calves under 1 year of age. In 2018, all 28 MSs, as well as Iceland, Republic of North Macedonia, Norway and Switzerland, reported data on fresh meat from broilers gathered at retail, whereas all 28 MSs as well as Iceland, Norway and Switzerland, reported data on broilers, and 10 MSs and Norway and Switzerland, on fattening turkeys.

Isolates were sampled through harmonised national schema. Microbroth dilution testing methods were used for susceptibility testing, and quantitative[2] isolate-based data were reported to EFSA and considered for this report. Resistance was interpreted using EUCAST ECOFF values (see following text box for further information). The antimicrobials incorporated in this summary analysis were selected based on their public health relevance and as representatives of different antimicrobial classes.

Data on *C. coli* in fattening pigs and calves and *C. jejuni* in calves, as well as data on meticillin-resistant *Staphylococcus aureus* (MRSA) and on specific monitoring of carbapenemase-producing microorganisms were reported on a voluntary basis.

2.1.1. Harmonised representative sampling and monitoring

Representative sampling should be performed according to general provisions of the legislation and to detailed technical specifications issued by EFSA (EFSA, 2014).

Salmonella spp.

In 2019, representative *Salmonella* isolates for monitoring AMR were collected by MSs from carcases of fattening pigs sampled for testing and verification of compliance, in accordance with point 2.1.4 of Chapter 2 of Annex I to Regulation (EC) No 2073/2005[3]; as well as carcases of bovines under 1 year of age where the production of meat of those bovines in the MSs is more than 10,000 tonnes slaughtered per year sampled for testing and verification of compliance, in accordance with point 2.1.3 of Chapter 2 of Annex I to Regulation (EC) No 2073/2005. MSs sampled carcases of fattening pigs/carcases of bovines under 1 year of age of healthy slaughter at the slaughterhouse. A two-stage stratified sampling design, with slaughterhouses as primary sampling units and carcases as secondary units, with

[1] Commission Implementing Decision 2013/652/EU of 12 November 2013 on the monitoring and reporting of antimicrobial resistance in zoonotic and commensal bacteria. OJ L 303, 14.11.2013, p. 26–39.
[2] 'Quantitative data' derived from dilution methods consisted of the number of isolates having a specific MIC value (measured in mg/L) relative to the total number of isolates tested, for each antimicrobial agent and specific food/animal category.
[3] Commission Regulation (EC) No 2073/2005 of 15 November 2005 on microbiological criteria for foodstuffs. OJ L 338, 22.12.2005, p. 1–26.

proportional allocation of the number of samples to the annual throughput of the slaughterhouse, was applied in the reporting countries.

In 2018, representative *Salmonella* isolates for monitoring AMR were collected by MSs from the populations of laying hens, broilers and fattening turkeys sampled according to the *Salmonella* National Control Programmes (NCPs), set up in accordance with Article 5(1) of Regulation (EC) No 2160/2003[4], as well as from carcasses of both broilers and fattening turkeys sampled for testing and verification of compliance, in accordance with point 2.1.5 of Chapter 2 of Annex 1 to Regulation (EC) No 2073/2005.

Not more than one isolate per *Salmonella* serovar from the same epidemiological unit (herd/holding/flock of birds) per year should be included in the AMR monitoring. In most MSs, the isolates tested for antimicrobial susceptibility constituted a representative subsample of the total *Salmonella* isolates available at the National Reference Laboratory (NRL) and/or other laboratories involved, obtained in a way that ensured geographical representativeness and even distribution over the year. Conversely, for low prevalence, all the *Salmonella* isolates available should be tested for susceptibility.

2.1.2. *Campylobacter* and indicator commensal *E. coli*[5]

Routine monitoring of indicator E. coli

In 2019, MSs collected indicator *E. coli* isolates as part of their national monitoring programme of AMR according to the provisions of the Decision 2013/652/EU, based on random sampling of caecal samples gathered at slaughter from fattening pigs and calves under 1 year of age where the production of meat of those bovines in the MSs is more than 10,000 tonnes slaughtered per year. Only one representative caecal sample (single or pooled) per epidemiological unit (batch of carcases deriving from the same herd), was gathered to account for clustering. Isolates were recovered from caecal contents samples (single or pooled), in accordance with EFSA's recommendations (EFSA, 2014). MSs shall test 170 isolates for antimicrobial susceptibility testing for each of animal population listed above. However, in MSs with a production of less than 100,000 tonnes of pig meat slaughtered per year they shall test 85 isolates instead of 170 isolates. The sample collection was approximately evenly distributed over the year 2019.

In 2018, MSs collected *Campylobacter jejuni* and indicator commensal *E. coli* isolates as part of their national monitoring programme of AMR according to the provisions of Commission Implementing Decision 2013/652/EU, based on representative random sampling of carcasses of healthy slaughter broilers/fattening turkeys at the slaughterhouse. A two-stage stratified sampling design, with slaughterhouses as primary sampling units and carcasses as secondary units, with proportional allocation of the number of samples to the annual throughput of the slaughterhouse, was applied in the reporting countries. Only one representative caecal sample (single or pooled) per epidemiological unit (batch of carcasses deriving from the same flock), was gathered to account for clustering. Isolates were recovered from caecal contents samples (single or pooled), in accordance with EFSA's recommendations (EFSA, 2014). The sample collection was approximately evenly distributed over the year 2018.

Specific monitoring of E. coli *ESBL/AmpC/carbapenemase producers*

In 2019, caecal samples gathered at slaughter from fattening pigs and bovines under 1 year of age, where the production of meat of those bovines in the MSs is more than 10,000 tonnes slaughtered per year and samples of fresh pig meat and bovine meat gathered at retail were collected. In 2018 caecal samples gathered at slaughter from broilers and from fattening turkeys, in those MSs where the production of turkey meat in the MS is more than 10,000 tonnes slaughtered per year, and samples of fresh meat from broilers gathered at retail were collected. Only one representative caecal sample (single or pooled) per epidemiological unit (batch of carcases deriving from the same herd/flock), was gathered to account for clustering. Isolates were recovered from caecal contents samples (single or pooled), in accordance with EFSA's recommendations (EFSA, 2014). MSs shall analyse 300 samples of each of the animal population and food category, listed in above. However, in MSs with a production of less than 100,000 tonnes of pig meat slaughtered per year, less than 50,000 tonnes bovine meat slaughtered

[4] Regulation (EC) No 2160/2003 of the European Parliament and of the Council of 17 November 2003 on the control of salmonella and other specified food-borne zoonotic agents. OJ L 325, 12.12.2003, p. 1–15.

[5] The same sampling design was used to collect indicator *E. coli* isolates, whether dedicated to the routine monitoring of AMR or the specific monitoring of ESBL-/AmpC-/carbapenemase-producing *E. coli*.

per year, and less than 100,000 tonnes of poultry meat slaughtered per year, the MS shall analyse 150 samples instead of 300 samples for each corresponding specific combination. The sample collection was approximately evenly distributed over the year 2019 and 2018 as described above.

2.1.3. Epidemiological cut-off values (ECOFFs) and clinical breakpoints (CBPs)

Epidemiological cut-off values (ECOFFs) and clinical breakpoints (CBPs)

A microorganism is defined as 'clinically' resistant when the degree of resistance shown is associated with a high likelihood of therapeutic failure. The microorganism is categorised as resistant by applying the appropriate CBP in a defined phenotypic test system, and this breakpoint may alter with legitimate changes in circumstances (for example alterations in dosing regimen, drug formulation, patient factors). A microorganism is defined as wild type for a bacterial species when no acquired or mutational resistance mechanisms are present to the antimicrobial in question. A microorganism is categorised as wild type for a given bacterial species presenting a lower MIC to the antimicrobial in question than the appropriate ECOFF in a defined phenotypic test system. This cut-off value will not be altered by changing circumstances (such as alterations in frequency of antimicrobial administration). Wild-type microorganisms may or may not respond clinically to antimicrobial treatment. A microorganism is defined as non-wild type for a given bacterial species by the presence of an acquired or mutational resistance mechanism to the antimicrobial in question. A microorganism is categorised as non-wild type for a given bacterial species by applying the appropriate ECOFF value in a defined phenotypic test system; non-wild-type organisms are considered to show 'microbiological' resistance (as opposed to 'clinical' resistance). CBPs and ECOFFs may be the same, although it is often the case that the ECOFF is lower than the CBP. EUCAST has defined CBPs and ECOFFs.

Clinical breakpoints (clinical resistance)

The clinician, or veterinarian, choosing an antimicrobial agent to treat humans or animals with a bacterial infection requires information that the antimicrobial selected is effective against the bacterial pathogen. Such information will be used, together with clinical details such as the site of infection, ability of the antimicrobial to reach the site of infection, formulations available and dosage regimes, when determining an appropriate therapeutic course of action. The in vitro susceptibility of the bacterial pathogen can be determined and CBPs used to ascertain whether the organism is likely to respond to treatment. CBPs will take into account the distribution of the drug in the tissues of the body following administration and assume that a clinical response will be obtained if the drug is given as recommended and there are no other adverse factors which affect the outcome. Conversely, if the CBP indicates resistance, then it is likely that treatment will be unsuccessful. Frequency of dosing is one factor that can affect the antimicrobial concentration achieved at the site of infection. Therefore, different dosing regimens can lead to the development of different CBPs, as occurs in some countries for certain antimicrobials where different therapeutic regimes are in place. Although the rationale for the selection of different CBPs may be clear, their use makes the interpretation of results from different countries in reports of this type problematic, as the results are not directly comparable between those different countries.

Epidemiological cut-off values (microbiological resistance)

For a given bacterial species, the pattern of the MIC distribution (i.e. the frequency of occurrence of each given MIC plotted against the MIC value) can enable the separation of the wild-type population of microorganisms from those populations that show a degree of acquired resistance. The wild-type susceptible population is assumed to have no acquired or mutational resistance and commonly shows a normal distribution. When bacteria acquire resistance by a clearly defined and efficacious mechanism, such as the acquisition of a plasmid bearing a gene which produces an enzyme capable of destroying the antimicrobial, then the MIC commonly shows two major subpopulations, one a fully susceptible normal distribution of isolates and the other a fully resistant population which has acquired the resistance mechanism. Resistance may be achieved by a series of small steps, such as changes in the permeability of the bacterial cell wall to the antimicrobial or other mechanisms which confer a degree of resistance. In this case, there may be populations of organisms which occur lying between the fully susceptible population and more resistant populations. The ECOFF value indicates the MIC or zone diameter above which the pathogen has some detectable reduction in susceptibility. ECOFFs are derived by testing an adequate number of isolates to ensure that the wild-type population can be confidently identified for a given antimicrobial. The clinical breakpoint, which is set to determine the therapeutic effectiveness of the antimicrobial, may fail to detect emergent resistance. Conversely, the ECOFF detects any deviation in susceptibility from the wild-type population, although it may not be appropriate for determining the likelihood of success or failure for clinical treatment.

2.1.4. *Campylobacter coli*

Caecal samples gathered at slaughter from fattening pigs were collected on a voluntary basis. One representative caecal sample (single or pooled) per epidemiological unit (batch of carcases deriving from the same herd), was gathered to account for clustering. Isolates were recovered from caecal contents samples (single or pooled), in accordance with EFSA's recommendations (EFSA, 2014). The sample collection was approximately evenly distributed over the year 2019.

2.1.5. MRSA

Isolates may have been collected by different monitoring approaches, either by active monitoring of animals and foods or, in some cases, by passive monitoring based on diagnostic submission of samples from clinical cases of disease in animals, or from foods sampled as part of investigatory work.

2.1.6. Harmonised antimicrobial susceptibility testing

Routine monitoring antimicrobial susceptibility

MSs tested antimicrobials and interpreted the results using the epidemiological cut-off values and concentration ranges shown in Table 5: and Table 6: to determine the susceptibility of *Salmonella* spp., *C. coli*, *C. jejuni* and indicator commensal *E. coli.* All *E. coli* isolates, randomly selected isolates of *Salmonella* spp. and *E. coli* that, after testing with the first panel of antimicrobials in accordance with Commission Implementing Decision 2013/652/EU were found to be resistant to cefotaxime, ceftazidime or meropenem, were further tested with a second panel of antimicrobial substances as shown in Table 7: . This panel notably includes cefoxitin, cefepime and clavulanate in combination with cefotaxime and ceftazidime for the detection of presumptive ESBL and AmpC producers, as well as imipenem, meropenem and ertapenem to phenotypically identify presumptive carbapenemase producers.

Specific monitoring of ESBL-/AmpC-/carbapenemase-producing E. coli

For the specific monitoring of ESBL-/AmpC-/carbapenemase-producing *E. coli*, the isolation method started with a non-selective pre-enrichment step, followed by inoculation on MacConkey agar containing a third-generation cephalosporin in a selective concentration (cefotaxime 1 mg/L), in accordance with the most recent version of the detailed protocol for standardisation of the EU Reference Laboratory for Antimicrobial Resistance (EURL-AR).[6] Using this protocol, also carbapenemase-producing isolates can be recovered. If available, one presumptive ESBL-/AmpC-/carbapenemase-producing *E. coli* isolate obtained from each positive caecal sample and meat sample was tested for its antimicrobial susceptibility to the first panel of antimicrobials (Table 5:) to confirm the microbiological resistance to cefotaxime (expected as the antimicrobial is present in the isolation medium at a concentration higher than the ECOFF), and identify possible resistance to ceftazidime and/or ceftazidime and/or meropenem. In a second step, the isolate should be tested using the second panel of antimicrobials (Table 7:) to infer the presumptive ESBL-/AmpC-/carbapenemase-producing phenotype according to the β-lactam resistance phenotype obtained (Figure 1:).

Specific monitoring of carbapenemase-producing microorganisms

This monitoring programme was performed and reported on a voluntary basis. For the specific monitoring of carbapenemase-producing microorganisms, isolation required the use of non-selective pre-enrichment and subsequent selective plating on carbapenem-containing media, in accordance with the most recent version of the detailed protocol of the EURL-AR. The microbial species was identified using an appropriate method. If available, one presumptive carbapenemase-producing isolate (primarily *E. coli*, but also *Salmonella*) obtained from each positive caecal sample and meat sample should be tested for its antimicrobial susceptibility to the first panel of antimicrobials (Table 5:) to confirm the microbiological resistance to meropenem and identify possible resistance to cefotaxime and/or ceftazidime. In a second step, the isolate should be tested using the second panel of antimicrobials (Table 7:) to infer the presumptive carbapenemase-producer phenotype according to the β-lactam resistance phenotype obtained (Figure 1:). The EUCAST epidemiological cut-off values applied for the

[6] Available online: www.eurl-ar.eu

antimicrobial susceptibility testing (Tables 3–5) are the ones available during the drafting of the Decision 2013/652/EU. For some antimicrobials, these values have been updated by EUCAST (www.eucast.org, last accessed 09.01.2020). Currently, for *Salmonella*, there is no ECOFF available anymore for colistin, tigecycline, nor ertapenem; for *E. coli*, there is no tigecycline nor ertapenem ECOFFs available anymore (additional updates, for *E. coli*, for temocillin the current value is 16 mg/L, and for cefotaxime/clavulanic acid and ceftazidime/clavulanic acid, 0.25 and 0.5 mg/L, respectively; for both *Salmonella* spp., and *E. coli*, the current ECOFFs for nalidixic acid is 8 mg/L). To allow comparison with the data collected in previous years, the ECOFFs laid down in the legislation are considered.

Table 5: Panel of antimicrobial substances included in AMR monitoring, EUCAST ECOFFs and concentration ranges tested in *Salmonella* spp. and indicator commensal *E. coli* (first panel) as laid down in Commission Implementing Decision 2013/652/EU

Antimicrobial	*Salmonella* EUCAST ECOFF[(a)]	*E. coli* EUCAST ECOFF[(a)]	Concentration range, mg/L (no. of wells)
Ampicillin	> 8	> 8	1–64 (7)
Cefotaxime	> 0.5	> 0.25	0.25–4 (5)
Ceftazidime	> 2	> 0.5	0.5–8 (5)
Meropenem	> 0.125	> 0.125	0.03–16 (10)
Nalidixic acid	> 16	> 16	4–128 (6)
Ciprofloxacin	> 0.064	> 0.064	0.015–8 (10)
Tetracycline	> 8	> 8	2–64 (6)
Colistin	> 2	> 2	1–16 (5)
Gentamicin	> 2	> 2	0.5–32 (7)
Trimethoprim	> 2	> 2	0.25–32 (8)
Sulfamethoxazole	NA[(b)]	> 64	8–1,024 (8)
Chloramphenicol	> 16	> 16	8–128 (5)
Azithromycin	NA[(c)]	NA[(c)]	2–64 (6)
Tigecycline	> 1	> 1	0.25–8 (6)

AMR: antimicrobial resistance; ECOFFs: epidemiological cut-off values; EUCAST: European Committee on Antimicrobial Susceptibility Testing; NA: not available.

(a): EUCAST epidemiological cut-off values available in Decision 2013/652/EU was drafted (2013). ">" than the ECOFF, criteria used for determing microbiological resistance.

(b): > 256 mg/L was used.

(c): > 16 mg/L was used.

Table 6: Panel of antimicrobial substances included in AMR monitoring, EUCAST ECOFFs and concentration ranges tested in *C. jejuni* and *C. coli*

Antimicrobial	*C. jejuni* EUCAST ECOFF[(a)]	*C. coli* EUCAST ECOFF[(a)]	Concentration range, mg/L (no. of wells)
Erythromycin	>4	> 8	1–128 (8)
Ciprofloxacin	>0.5	> 0.5	0.12–16 (8)
Tetracycline	>1	> 2	0.5–64 (8)
Gentamicin	>2	> 2	0.12–16 (8)
Nalidixic acid	>16	> 16	1–64 (7)
Streptomycin[(b)]	>4	> 4	0.25–16 (7)

AMR: antimicrobial resistance; EUCAST: European Committee on Antimicrobial Susceptibility Testing; ECOFFs: epidemiological cut-off values; NA: not available.

(a): EUCAST epidemiological cut-off values. ">" than the ECOFF, criteria used for determing microbiological resistance.

(b): On a voluntary basis.

Table 7: Panel of antimicrobial substances, EUCAST ECOFFs and concentration ranges used for testing only *Salmonella* spp. and indicator commensal *E. coli* isolates resistant to cefotaxime, ceftazidime or meropenem (second panel)

Antimicrobial	*Salmonella* EUCAST ECOFF[(a)]	*E. coli* EUCAST ECOFF[(a)]	Concentration range, mg/L (no. of wells)
Cefoxitin	> 8	> 8	0.5–64 (8)
Cefepime	NA[(b)]	> 0.125	0.06–32 (10)
Cefotaxime + clavulanic acid	NA	NA	0.06–64 (11)
Ceftazidime + clavulanic acid	NA	NA	0.125–128 (11)
Meropenem	> 0.125	> 0.125	0.03–16 (10)
Temocillin	NA[(d)]	NA[(d)]	0.5–64 (8)
Imipenem	> 1	> 0.5	0.12–16 (8)
Ertapenem	> 0.06	> 0.06	0.015–2 (8)
Cefotaxime	> 0.5	> 0.25	0.25–64 (9)
Ceftazidime	> 2	> 0.5	0.25–128 (10)

ECOFFs: epidemiological cut-off values; EUCAST: European Committee on Antimicrobial Susceptibility Testing; NA: not available.

(a): EUCAST epidemiological cut-off values available as the Decision 2013/652/EU was drafted (2013). For some antimicrobials, these values have been updated (see below). ">" than the ECOFF, criteria used for determing microbiological resistance.

(b): > 0.125 mg/L was used.

(c): Current ECOFFs 0.25 and 0.5 mg/L. respectively.

(d): For temocillin the cut-off value used in the analysis was > 32 mg/L.

2.2. Data validation

2.2.1. Validation against business rules

The reported data were first checked for usability against a series of 'business rules', which were automatically applied in the EFSA data collection system once a file was sent. This automatic data validation process refers to the first validation of incoming data. Quality checks are related to a specific business only. The positive result of the automatic validation process places the file in a valid state and makes it available for further steps of validation performed by EFSA.

2.2.2. Scientific data validation

The scientific validation of the data collected by the MSs/non-MSs and submitted to EFSA consisted on the revision of data and comparison between data reported for the same antimicrobials when tested by different panels. Special attention was given to carbapenems, colistin, azithromycin, tigecycline and to possible discrepancies between results for antimicrobials present in both panels (i.e. cefotaxime, ceftazidime, meropenem). MSs were contacted by EFSA asking for clarifications. If considered needed, MSs were asked to confirm the MIC results and the species identification of the reported isolates.

2.2.3. Reference testing

To ensure the quality of data submitted, a reference testing exercise was run by the EURL-AR in close collaboration with the MSs. The exercise consisted in retesting the AST of the isolates received using both Panel 1 and Panel 2 of antimicrobials, as well as whole genome sequencing (WGS) analyses of the isolates (WGS analyses on-going by the time of drafting the present report). Based on the data submitted to EFSA, a selection of approximately 400 isolates/per year was made. The selection of these isolates was based on different criteria:

- The EURL-AR had reported technical issues when testing azithromycin, tigecycline and colistin during the EURL workshop hold in Lyngby (Denmark) in 2016 (www.eurl-ar.eu). Resistant isolates from countries with outstanding prevalence for these antimicrobials were asked to provide selected isolates to the EURL-AR. Most of the *E. coli* isolates chosen were selected among the ones reported mainly for the specific ESBL/AmpC/carbapenemase monitoring.
- There was a discrepancy between MIC values reported for the antimicrobials present in both panels (impacting the categorisation of the isolate as resistant or susceptible).

- If according to the criteria applied (2.5.1), the presence of carbapenemase producers was suspected.
- Isolates representing the categorisations presumptive ESBLs-, AmpC and ESBL + AmpC producers.
- Isolates with odd phenotypes.
- Selected multi-drug resistant isolates from specific *Salmonella* serotypes that could represent widespread clones.
- Isolates microbiologically resistant to ciprofloxacin and susceptible to nalidixic acid (presence of plasmid mediated quinolone resistance encoding genes, PMQR, suspected) were included in the selection.

The MSs/non-MSs sent the selected isolates to the EURL-AR, where they were retested. EFSA, EURL-AR and MSs liaised together to address possible discrepancies found.

2.3. Analyses of antimicrobial resistance data

Data are reported in separate sections dedicated to each microorganism. Clinical investigation data were not accounted for in this report.

2.3.1. Overview tables of the resistance data reported

Data generated from the antimicrobial susceptibility testing and reported as quantitative at the isolate level by MSs have been described in the overview tables published on the EFSA website (see Annex B-F). The tables also display complete susceptibility, multidrug resistance and co-resistance. These analyses are described in Section 2.4.

2.3.2. Minimum inhibitory concentration distributions

For each combination of microorganism, antimicrobial and food category/animal population were tested, MIC distributions were tabulated in frequency tables, giving the number of isolates tested that have a given MIC at each test dilution (mg/L) of the antimicrobial. Isolate-based dilution results allowed MIC distributions reported:

- for *Salmonella* for ampicillin, azithromycin, cefepime, cefotaxime, cefotaxime and clavulanic acid, ceftazidime, ceftazidime and clavulanic acid, cefoxitin, chloramphenicol, ciprofloxacin, colistin, ertapenem, gentamicin, imipenem, meropenem, nalidixic acid, sulfamethoxazole, temocillin, tetracycline, tigecycline and trimethoprim;
- for *Campylobacter* for ciprofloxacin, erythromycin, gentamicin, nalidixic acid, streptomycin and tetracycline;
- for indicator *E. coli* for ampicillin, azithromycin, cefepime, cefotaxime, cefotaxime and clavulanic acid, ceftazidime, ceftazidime and clavulanic acid, cefoxitin, chloramphenicol, ciprofloxacin, colistin, ertapenem, gentamicin, imipenem, meropenem, nalidixic acid, sulfamethoxazole, temocillin, tetracycline, tigecycline and trimethoprim;
- for MRSA for cefoxitin, chloramphenicol, ciprofloxacin, clindamycin, erythromycin, fusidic acid, gentamicin, kanamycin, linezolid, mupirocin, penicillin, quinupristin/dalfopristin, rifampicin, streptomycin, sulfamethoxazole, tetracycline, tiamulin, trimethoprim and vancomycin.

2.3.3. Epidemiological cut-off values and the occurrence of resistance

ECOFFs, as listed in Decision 2013/652/EC, have been used in this report to interpret the isolate-based reported MIC data and determine non-wild-type organisms also termed 'microbiologically' resistant organisms (i.e. displaying a decreased susceptibility), and to ensure that results from different MSs are comparable. From this point onwards in this report, 'microbiologically' antimicrobial-resistant organisms are referred to as 'resistant' for brevity. This report also incorporates re-evaluation of the historical data accounting for the revised EU legislation, which included the revised ECOFFs.

The occurrence of resistance[7] to a number of antimicrobials was determined for *Salmonella*, *Campylobacter*, and indicator commensal *E. coli* isolates and are tabulated at the production-type level in this report. The occurrence of resistance (i.e. resistance levels) in reporting MS groups was calculated as totals (the total number of resistant isolates out of the total number of tested isolates across reporting MSs) and in the *E. coli* chapter, also as weighted means to account for the animal population sizes.

2.3.4. Data description

Throughout the report, level or occurrence of AMR means the percentage of resistant isolates as a proportion of the isolates tested of that microorganism. MSs reporting group means the MSs that provided data and were included in the relevant table of antimicrobial resistance for that bacterium–food or animal category–antimicrobial combination. Terms used to describe the levels or occurrence of antimicrobial resistance are 'rare': < 0.1%, 'very low': 0.1–1.0%, 'low': > 1–10.0%, 'moderate': > 10.0–20.0%, 'high': > 20.0–50.0%, 'very high': > 50.0–70.0%, 'extremely high': >70.0%. Although these terms are applied to all antimicrobials, the significance of a given level of resistance depends on the particular antimicrobial and its importance in human and veterinary medicine.

2.3.5. Temporal trends in resistance

Where the minimum criteria for data inclusion in this report were met, temporal trend graphs were generated to show the resistance to different antimicrobials from 2009 to 2019, by plotting the level of resistance for each year of sampling. Graphs were created for those countries for which resistance data were available for three or more years in the 2009–2019 period. MS-specific resistance levels trend graphs use a unique scale and countries are shown in alphabetical order. For ampicillin, cefotaxime, ciprofloxacin, and tetracyclines (*Salmonella* and indicator *E. coli*), ciprofloxacin, erythromycin, streptomycin and tetracycline (*Campylobacter*), resistance trends over time were visually explored by trend graphs, produced using SAS® Studio.

To assess the statistical significance of temporal trends, the proportions of resistance were modelled against time in a logistic regression. This analysis was carried out using the PROC LOGISTIC of SAS 9.4 for each country reporting at least 10 total tested isolates, where there were 3 years or more of available data to use in the model. The PROC LOGISTIC function uses a logit transformation to model the proportion of prevalence against year, and provides estimates for both intercepts and slope. Models where the likelihood ratio test suggested it to be meaningful and resulting in a p-value associated with slope of < 0.05 were considered to be significant (linear model fit). It is important to note that between-year fluctuations in the occurrence resistance (%) may not be captured in the evaluation of the trend over the entire time period (2009-2019) and that very recent decreasing or increasing trends may therefore be masked by the overall trend.

2.3.6. Spatial analysis of resistance through maps

MS-specific AMR levels for selected bacterium–food category/animal population combinations were plotted in maps for 2018 and 2019, using ArcGIS 9.3. In the maps, resistance levels are presented with colours reflecting the continuous scale of resistance to the antimicrobial of interest among reporting MSs; so, there might be some apparent discrepancies between the colours and resistance levels between maps.

2.3.7. Resistance in *Salmonella* serovars of public health importance

In this report, AMR in tested *Salmonella* isolates were aggregated to give a value for *Salmonella* spp. for each country and food/animal category. In addition, the most prevalent *Salmonella* serovars were also reported separately for particular food/animal category. Additional tables have been included in this report to describe the occurrence of AMR among selected *Salmonella* serovars of public health relevance or of marked prevalence in animals. To present a complete overview of the animal populations and food categories in which specific *Salmonella* serovars of public health importance have been recovered, all the data reported (derived even from fewer than 4 reporting countries and less than 10 isolates tested) have been included.

[7] Giving the percentage of isolates 'microbiologically' resistant out of those tested.

2.4. Analysis of multidrug resistance, complete susceptibility and co-resistance data

The analysis of MDR and co-resistance data is important in light of the emergence of multiresistant bacteria. The intention is to focus mainly on multi/co-resistance patterns involving critically important antimicrobials (WHO, 2019), such as cephalosporins, fluoroquinolones and macrolides, and to summarise important information in the EU Summary Report. The occurrence of the isolates of a serotype/resistance pattern of interest is studied both at the MS level and at the EU level (by grouping data for all MSs and where also relevant for MSs and other reporting countries), as the overall picture for all MSs might show a more definite pattern of emergence and spread. In addition, the analysis of data may reveal the existence of new or emerging patterns of MDR, particularly in *Salmonella* serotypes.

2.4.1. Analysis of MDR and complete susceptibility

For the analysis of MDR and complete susceptibility, a multiresistant isolate is one defined as resistant to at least three of the antimicrobial substances that should be included in the AMR monitoring according to Commission Implementing Decision 2013/652/EU (see Table 5: and Table 6: In contrast, a completely susceptible isolate is one defined as non-resistant (MIC<ECOFF) to these antimicrobial substances. Resistance to nalidixic acid and resistance to ciprofloxacin, as well as the resistance to cefotaxime and to ceftazidime are, respectively, addressed together. Due to the presence of resistance to colistin considered as intrinsic in serogroup D of *Salmonella* spp., colistin was not included in the analysis of MDR and complete susceptibility for *Salmonella*. MDR and completely susceptibility are visually displayed in "traffic light graphs".

For indicator *E. coli*, the occurrence of complete susceptibility (OI_{CS}) is also displayed in bar charts showing the trends for the years 2015, 2017 and 2019 for porcine and bovine populations and for the years 2014, 2016 and 2018 for poultry populations, respectively. The statistical significance of the trends was analysed using chi-squared tests for trends. The rate of change (ROC) (expressed in percent) is shown for significant temporal trends of OI_{CS}. It is used to mathematically describe the percentage change in value of OI over a defined period of time. It represents the momentum of the OI. The calculation for ROC takes the last value of OI and divides it by the initial measurement. One is subtracted from this value and the resulting number is multiplied by 100 to give it a percentage representation.

For *Campylobacter*, a MDR isolate is one defined as resistant to at least three of the antimicrobial substances included in the AMR monitoring according to Commission Implementing Decision 2013/652/EU (see table 2 of this Decision), except for streptomycin. In contrast, a completely susceptible isolate is one defined as non-resistant (MIC< or equal to ECOFF) to the panel of antimicrobial substances described in the Decision, excluding streptomycin. As streptomycin is not used in humans, its exclusion in the analysis of MDR and complete susceptibility for *Campylobacter* has been agreed by EFSA and ECDC to allow comparability of MDR and CS in humans and food-producing animals.

2.4.2. MDR patterns

The frequency and percentage of isolates exhibiting various MDR patterns considering the antimicrobials tested were determined for *Salmonella* (*Salmonella* spp. and for certain serovars of interest), *Campylobacter* species and indicator *E. coli* for each country and each animal population/food category. Isolates for which no susceptibility data were provided for some of the antimicrobial substances, were disregarded.

2.4.3. 'Key Outcome Indicators'

To support EU countries in their progress to reduce use of antimicrobials and AMR a list of key outcome indicators has been jointly published by ECDC, EFSA and EMA (ECDC, EFSA and EMA, 2017). Two of these key outcome indicators (KOI) are included in the report: (1) The key outcome indicator of complete susceptibility (KOI_{CS}) in indicator *E. coli*; and (2) the key outcome indicator of the prevalence of ESBL- and/or AmpC-producing E. coli (KOI_{ESC}).

KOI_{CS} is the proportion of fully susceptible indicator *E. coli* isolates, weighted by the size of the populations of the most important production animals (broilers, fattening turkeys, fattening pigs, calves) and is used as an indicator (KOI_{CS}) for the overall AMR situation in food-producing animals. KOI_{ESC} is the

weighted mean of the prevalence of ESBL- and/or AmpC-producing *E. coli* in each of the four animal populations monitored. The identification of presumptive ESBL and AmpC producers is described in 2.5. The KOI_{CS} and KOI_{ESC} account for differences in the relative size of food animal populations in a country and are therefore relevant in evaluation of risks related to resistance in food animals.

These KOIs are displayed in bar charts showing changes in KOI over the 2014-2019 period for OI_{CS} in indicator E. coli and 2015-2019 period for OI_{ESC}. The statistical significance of the trends was analysed using chi-squared tests for trends. The rate of change (ROC) (expressed in percent) is shown for significant temporal trends of KOI_{CS} and KOI_{ESC}. It is used to mathematically describe the percentage change in value of KOI over a defined period of time. It represents the momentum of the KOI. The calculation for ROC takes the last value of KOI and divides it by the initial measurement. One is subtracted from this value and the resulting number is multiplied by 100 to give it a percentage representation.

2.4.4. The co-resistance patterns of interest

The term combined resistance is used in this report to indicate phenotypic resistance to two or more different classes of antimicrobials, exhibited by the same bacterial isolate. In *Salmonella* and *E. coli* isolates, co-resistance to cefotaxime (CTX) and ciprofloxacin (CIP) was estimated, as these two antimicrobials are of particular interest in human medicine. Co-resistance was addressed using both ECOFFs (CTX > 0.25 mg/L and CIP > 0.064 mg/L) and CBPs (CTX > 2 mg/L and CIP > 1 mg/L) for *E. coli*. In *C. jejuni* and *C. coli* isolates, co-resistance to ciprofloxacin and erythromycin (ERY) was estimated, as these two antimicrobials are of particular interest in human medicine in the treatment of severe campylobacteriosis. The interpretive ECOFFs used to address co-resistance to ciprofloxacin and erythromycin were, for *C. jejuni*, CIP > 0.5 mg/L and ERY > 4 mg/L and, for *C. coli*, CIP > 0.5 mg/L and ERY > 8 mg/L. These values may be considered as very similar to CBPs.

2.5. Identification of presumptive ESBL, AmpC and/or carbapenemase producers

2.5.1. Definition of ESBL, AmpC, ESBL+AmpC, CP-phenotypes:

The categorisation of isolates resistant to third-generation cephalosporins and/or carbapenems in presumptive ESBL, AmpC or carbapenemase producers was carried out based on the EUCAST guidelines for detection of resistance mechanisms and specific resistances of clinical and/or epidemiological importance (EUCAST, 2017). In these expert guidelines and, based on other EUCAST and CLSI guidelines to detect ESBL/AmpC producers, a screening breakpoint of > 1 mg/L is recommended for cefotaxime and ceftazidime. This screening breakpoint is higher than the ECOFFs applied for antimicrobial susceptibility of both antimicrobials for *E. coli*, and to cefotaxime for *Salmonella*. For this report, a first condition for classifying isolates as presumptive ESBL/AmpC producers related to their MIC for either cefotaxime or ceftazidime, was to apply this screening breakpoint of MICs > 1 mg/L. Only isolates which presented MIC values accomplishing with this requisite (as expected for most of the ESBL/AmpC producers) were further considered. In total, for the third generation cephalosporin- and/or carbapenem-resistant isolates, five main categorisations are made: 1. ESBL phenotype; 2. AmpC phenotype; 3. ESBL + AmpC phenotype; 4. CP-phenotype; and 5. Other phenotypes (Figure 1:).

1. To detect the production of ESBLs, a synergy test for cefotaxime and ceftazidime, in combination with clavulanic acid was performed. An eight-fold reduction in the MIC for the cephalosporin combined with clavulanic acid compared with that obtained for the cephalosporin alone was interpreted as a positive synergy test. In all other cases, the synergy test was considered negative. For the present report, isolates with MICs > 1 mg/L for cefotaxime and/or ceftazidime and a synergy test positive for any of these antimicrobials, together with susceptibility to cefoxitin (≤ 8 mg/L) and meropenem (MEM ≤ 0.125 mg/L see CP phenotype) were classified as **ESBL phenotype** (Figure 1:).
2. For the AmpC phenotype, the combination MIC > 8 mg/L (ECOFF) for cefoxitin together with MICs > 1 mg/L for cefotaxime and/or ceftazidime was used as phenotypic criteria to investigate the presence of AmpC production in *E. coli*. It should be also underlined that there are a few AmpC enzymes that do not confer resistance to cefoxitin (i.e. ACC-1), and that there are other

mechanisms (porin loss, presence of carbapenemases, a few ESBLs like cefotaxime (CTX-M-5) that could generate similar MIC values for the different antimicrobials (EFSA, 2012a; EUCAST, 2017). Phenotypic AmpC confirmation tests (i.e. cloxacillin synergy) were not required for the present monitoring. For the present report, isolates with MICs > 1 mg/L for cefotaxime and/or ceftazidime and cefoxitin MIC > 8 mg/L, together with negative synergy test for both cefotaxime and ceftazidime/clavulanic acid, together with susceptibility to meropenem (MEM ≤ 0.125 mg/L) were classified in the **AmpC phenotype** category. No distinction between acquired AmpC and natural AmpC was made (Figure 1:).

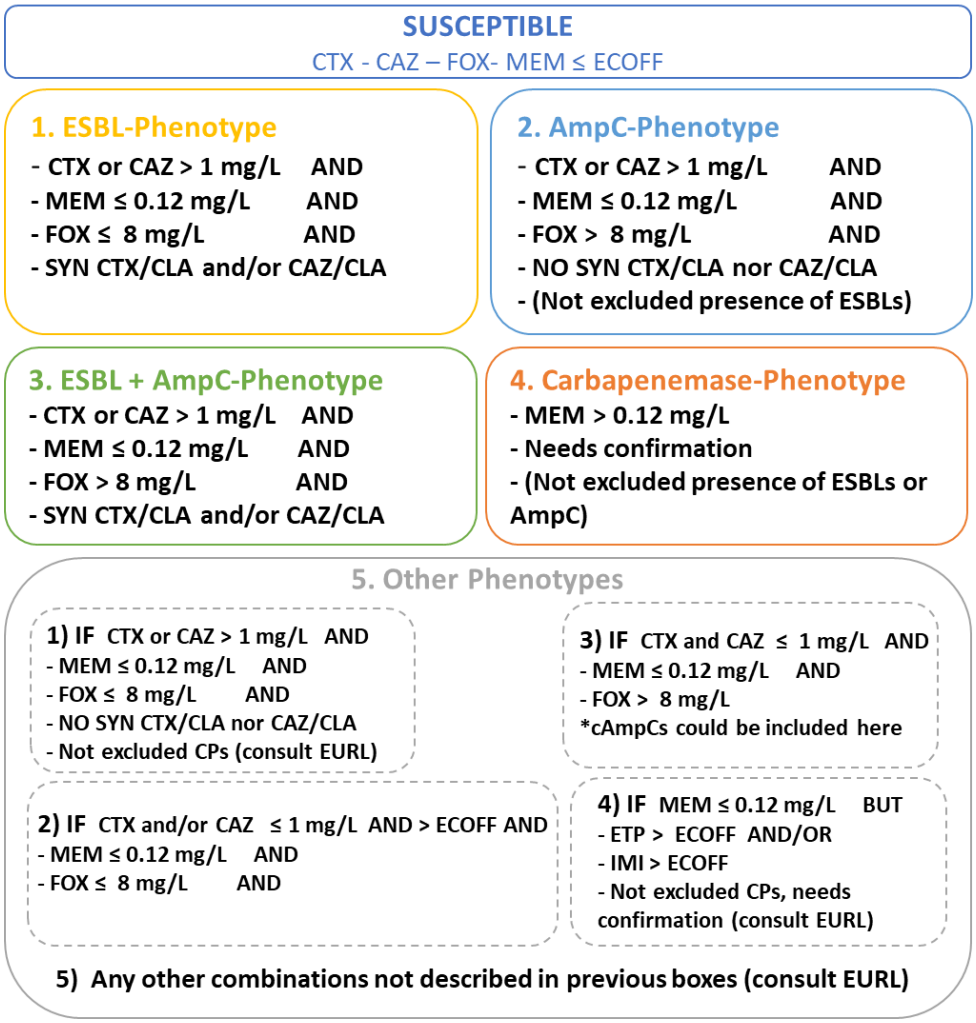
3. For the present report, isolates with MICs > 1 mg/L for cefotaxime and/or ceftazidime, positive synergy tests for any of these antimicrobials with clavulanic acid and cefoxitin MIC > 8 mg/L, together with susceptibility to meropenem (MEM ≤ 0.125 mg/L) were classified under the **ESBL + AmpC phenotype** category (Figure 1:).

   In some isolates, several mechanisms can be present at the same time, making it very difficult to differentiate the phenotypes. Also the high-level expression of AmpC β-lactamases can mask the presence of ESBLs. AmpC can also be present in isolates with positive ESBL tests (clavulanic acid synergy). In this case, the cefepime/clavulanic acid synergy test should be used to overturn/confirm the presence of ESBLs in these isolates (EUCAST, 2017) but, unfortunately, the combination cefepime/clavulanic acid was not included among the substances tested for monitoring. The inclusion of resistance to cefepime with a MIC value ≥ 4 mg/L as an additional criterion proposed elsewhere (EFSA, 2012), could be useful to ascertain the presence of an ESBL-producer.

4. For the classification of isolates into the putative **carbapenem producers (CPs)**, a meropenem screening cut-off of > 0.125 mg/L (which coincides with the harmonised ECOFF) was chosen. It is known that other mechanisms (i.e. hyperproduction or combination of ESBLs and/or AmpC and porin loss) can also affect to the MIC values generated for the different carbapenems, especially for ertapenem. The confirmation of the carbapenemase production recommended by the EUCAST guidelines cannot be inferred from the carbapenem susceptibility testing data reported but needs further phenotypic or molecular testing. Those MSs that reported data suggesting the presence of putative CPs were recommended to validate the results by performing further confirmatory testing, and the EURL-AR offered to apply WGS of the isolates. For the present report, isolates with MIC > 0.125 mg/L for meropenem would be considered as presumptive CP producers and were classified under the **CP phenotype**. The presence of other resistance mechanisms (ESBLs, AmpC, etc.) within the isolates placed in this group cannot be ruled out.

5. In this group, phenotypes not included in the categorisations defined above were included: isolates with a MIC > 0.125 for ertapenem and/or MIC > 1 mg/L for imipenem (EUCAST screening cut-offs, one dilution step higher than the currently defined ECOFFs) but no resistance to meropenem (MIC < 0.125 mg/L) were classified under the category 'other phenotype'. Finally, isolates with MICs ≤ 1 mg/L for cefotaxime and ceftazidime would be considered as not ESBL and/or AmpC producers. This implied that some isolates considered as microbiologically resistant (MICs over the ECOFFs) would not be further classified, as probably other mechanisms or technical issues in the MIC testing (i.e. MIC value close to the ECOFF) would be responsible for the MIC values obtained. For the present report, cefotaxime- and ceftazidime-resistant isolates with MICs ≤ 1 mg/L for both antimicrobials were considered as putative non-ESBL/AmpC producers and were classified under the category 'other phenotype'.

We are aware that without a further molecular characterisation of the isolates, it will not be possible to know exactly which resistance mechanisms are present. For epidemiological purposes and based on the EUCAST guidelines, the classification of 'presumptive' producers for the different mechanism conferring resistance to third-generation cephalosporins and/or carbapenems was considered. Molecular characterisation of these mechanisms is recommended.



Presumptive ESBL-producers include isolates exhibiting Phenotype 1 or 3.
Presumptive AmpC producers include isolates exhibiting Phenotype 2 or 3.

Figure 1: Phenotypes inferred based on the resistance to the β-lactams included in Panel 2

For the occurrence and prevalence tables, as well as the maps and graphics shown in Section 'ESBL/AmpC/CP producers monitoring', presumptive ESBL producers were considered as those exhibiting an ESBL and/or ESBL + AmpC phenotype, and presumptive AmpC producers, those with an AmpC and ESBL + AmpC phenotype (see below).

For the present report, the terms:

"Presumptive ESBL/AmpC producers" refers to those isolates who present an ESBL and/or and AmpC and/or an ESBL + AmpC phenotype (presumptive ESBL producers and/or presumptive AmpC producers).

"Presumptive ESBL producers" refers to those isolates isolates with MICs > 1 mg/L for cefotaxime and/or ceftazidime and a synergy test positive for any of these antimicrobials and susceptibility to meropenem (MEM ≤ 0.125 mg/L, see CP phenotype). These isolates may also harbour other resistance mechanisms (e.g. AmpC-encoding genes).

"Presumptive ESBL-cefotaximase producers" refers to those presumptive ESBL producers with MICs > 1 mg/L for cefotaxime and a synergy test positive for cefotaxime *only*. These isolates may also harbour other resistance mechanisms.

"Presumptive ESBL-ceftazidimase producers" refers to those presumptive ESBL producers with MICs > 1 mg/L for ceftazidime and synergy test positive for ceftazidime *only*. These isolates may also harbour other resistance mechanisms.

"**Presumptive AmpC producers**" refers to isolates with MICs > 1 mg/L for cefotaxime and/or ceftazidime and cefoxitin MIC > 8 mg/L together with susceptibility to meropenem (MEM ≤ 0.125 mg/L, see CP phenotype). No distinction between acquired AmpC and natural AmpC was made. These isolates may also harbour other resistance mechanisms (e.g. ESBL-encoding genes).

"**Presumptive ESBL + AmpC producers**" refers to isolates isolates with the ESBL + AmpC phenotype described above.

"**Presumptive carbapenemase-producers** (CP-producers)" refers to those isolates with the CP phenotype described above.

2.6. Data on meticillin-resistant *Staphylococcus aureus* (MRSA)

The occurrence of MRSA and its susceptibility to antimicrobials in various food categories (including meat samples from various species) and food-producing animals was reported by six MSs and two non-MSs in 2019 and in 2018 (excluding clinical investigations). In 2019, Finland and Switzerland were the only countries to report susceptibility data for MRSA isolates from meat samples (both countries also reported molecular typing data); Belgium and Switzerland were the only countries in 2019 to report such data for MRSA isolates from food-producing animals (both countries also reported molecular typing data, as did Finland, Norway and Spain). In 2018, Austria and Switzerland were the only countries to report susceptibility data on MRSA isolates from meat samples, with both countries additionally reporting molecular typing data; Belgium was the only country in 2018 to report susceptibility data on isolates from food-producing animals (and also provided molecular typing data, as did Denmark). MRSA occurrence data reported from clinical investigations of food-producing and companion animals in 2019/2018 were also reported. Details of the antimicrobials selected are provided in the section on MRSA. For further information on reported MIC distributions and the number of resistant isolates, refer to the submitted and validated MS data published on the EFSA website.

The methods for collecting and testing samples for MRSA are not harmonised between MSs and, as a result, MSs may use differing procedures. Due to the variety of methods employed by MSs, these are explained in detail within the section on MRSA to enable readers to better follow the procedures carried out by individual countries.

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