ANNEX

PART A

Sampling framework and analysis

1. Origin of bacterial isolates subject to antimicrobial susceptibility testing

Member States shall obtain bacterial isolates for AMR monitoring from at least each of the following combinations of isolates/food-producing animal populations/food:

- (a) Salmonella spp. isolates obtained from:
 - samples of each population of laying hens, broilers and fattening turkeys taken in the framework of the national control programmes provided for in Article 5 of Regulation (EC) No 2160/2003;
 - (ii) samples of caecal content taken at slaughter from fattening pigs, except for Member States implementing a national programme for the control of salmonella which has been approved at EU level;
 - (iii) samples of caecal content taken at slaughter from bovine animals under one year of age where the national production of meat of those bovine animals is more than 10 000 tonnes per year;
 - (iv) samples of fresh meat of broilers and turkeys taken at the border control posts.
- (b) *C. coli* and *C. jejuni* isolates obtained from
 - (i) samples of caecal content taken at slaughter from broilers;
 - samples of caecal content taken at slaughter from fattening turkeys where the national production of turkey meat is more than 10 000 tonnes per year;
 - (iii) samples of caecal content taken at slaughter from bovine animals under one year of age where the national production of meat of those bovine animals is more than 10 000 tonnes per year;
 - (iv) samples of caecal content taken at slaughter from fattening pigs.
- (c) Indicator commensal *E. coli* isolates obtained from:
 - (i) samples of caecal content taken at slaughter from broilers;
 - samples of caecal content taken at slaughter from fattening turkeys where the national production of turkey meat is more than 10 000 tonnes per year;
 - (iii) samples of caecal content taken at slaughter from fattening pigs;
 - samples of caecal content taken at slaughter from bovine animals under one year of age where the national production of meat of those bovine animals is more than 10 000 tonnes per year;
 - (v) samples of fresh meat of broilers, turkeys, pigs and bovine animals taken at the border control posts.
- (d) ESBL- or AmpC- or CP-producing *E. coli* isolates obtained from:

- (i) samples of caecal content taken at slaughter from broilers;
- (ii) samples of caecal content taken at slaughter from fattening turkeys where the national production of turkey meat is more than 10 000 tonnes per year;
- (iii) samples of caecal content taken at slaughter from fattening pigs;
- (iv) samples of caecal content taken at slaughter from bovine animals under one year of age where the national production of meat of those bovine animals is more than 10 000 tonnes per year;
- (v) samples of fresh meat of broilers, turkeys, pigs and bovine animals taken at retail;
- (vi) samples of fresh meat of broilers, turkeys, pigs and bovine animals taken at the border control posts.
- (e) Where a Member State decides to monitor indicator commensal *E. faecalis* and *E. faecium* in accordance with Article 1(3), isolates of these bacteria obtained from:
 - (i) samples of caecal content taken at slaughter from broilers;
 - samples of caecal content taken at slaughter from fattening turkeys where the national production of turkey meat is more than 10 000 tonnes per year;
 - (iii) samples of caecal content taken at slaughter from fattening pigs;
 - (iv) samples of caecal content taken at slaughter from bovine animals under one year of age where the national production of meat of those bovine animals is more than 10 000 tonnes per year.

2. Sampling frequency

Member States shall carry out the AMR monitoring of each combination of bacterial isolates/food-producing animal populations/food, as listed in point 1, in accordance with the following rotational system:

- (a) In the years 2021, 2023, 2025 and 2027: AMR monitoring shall be carried out in fattening pigs, bovine animals under one year of age, pig meat and bovine meat.
- (b) In the years 2022, 2024 and 2026: AMR monitoring shall be carried out in laying hens, broilers, fattening turkeys and fresh meat derived from broilers and turkeys.

3. Sampling design and sample size

- 3.1. At slaughterhouse level
- (a) Sampling design:

When designing their sampling plan at slaughterhouse level, Member States shall take into account EFSA technical specifications on randomised sampling for harmonised monitoring of antimicrobial resistance in zoonotic and commensal bacteria⁽¹⁾.

Member States shall ensure a proportionate stratified sampling of samples of caecal content in slaughterhouses processing at least 60 % of the specific domestic animal population in the Member States with an even distribution over the monitoring period of the samples taken, and, to the extent possible, a randomisation of the sampling days of each month. The samples shall be taken from healthy animals sampled from randomly selected epidemiological units. The

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epidemiological unit for broilers and fattening turkeys is the flock. The epidemiological unit for fattening pigs and bovine animals under one year of age is the slaughter batch. Only one sample from the same epidemiological unit shall be taken per year. Each sample shall be taken from one carcass randomly selected from the epidemiological unit. However, for broilers, each sample shall be taken from ten carcasses randomly selected from the epidemiological unit.

The number of samples collected per slaughterhouse shall be proportional to the annual throughput of each slaughterhouse covered by the sampling plan.

(b) Sample size:

In order to test for antimicrobial susceptibility the required minimum number of bacterial isolates referred to in point 4.1, Member States shall take annually a sufficient number of samples referred to in points 1(a)(ii) and (iii), 1(b) and 1(c)(i) to (iv) by accounting for the estimated prevalence of the bacterial species monitored in the animal population considered.

By way of derogation, when the prevalence of the bacterial species monitored is known to be inferior or equal to 30 % in the animal population considered or when this prevalence is unknown in the first year of the monitoring or when the number of epidemiological units available for sampling is insufficient to prevent the repeated sampling of the same units, Member States may decide to limit to 300 the annual number of samples to be taken. This annual number can be further reduced to 150 for each specific combination of bacterial isolates/animal populations where Member States have an annual national production of less than 100 000 tonnes of broiler meat, less than 100 000 tonnes of turkey meat, less than 100 000 tonnes of pig meat or less than 50 000 tonnes of bovine meat. Member States making use of the possibility of limitation of the annual number of samples shall base their decision on documented evidence, such as results of surveys, and shall submit this evidence to the Commission before implementing the reduced sampling for the first time.

Member States shall take annually at least 300 samples from each animal population referred to in points 1(d)(i) to (iv). By way of derogation, where Member States have an annual national production of less than 100 000 tonnes of broiler meat, less than 100 000 tonnes of turkey meat, less than 100 000 tonnes of pig meat or less than 50 000 tonnes of bovine meat, they may decide to take a minimum of 150 samples instead of 300 samples for each specific animal population considered.

3.2. At retail level

(a) Sampling design:

When designing their sampling plan at retail level, Member States shall take into account EFSA technical specifications on randomised sampling for harmonised monitoring of antimicrobial resistance in zoonotic and commensal bacteria⁽²⁾.

Member States shall ensure a proportionate stratified sampling of samples of the fresh meat taken at retail without pre-selecting samples based on the origin of the food, with a proportional allocation of the number of samples to the population of the geographical region. They shall also ensure an even distribution over the monitoring year of the samples of fresh meat and, to the extent possible, a randomisation of the sampling days of each month. The batches to be sampled on a given day shall be randomly selected.

(b) Sample size:

Member States shall take 300 samples from each fresh meat category referred to in point 1(d) (v). By way of derogation, where Member States have an annual production of less than 100 000 tonnes of broiler meat, less than 100 000 tonnes of turkey meat, less than 100 000 tonnes

of pig meat or less than 50 000 tonnes of bovine meat, they may decide to take 150 samples instead of 300 samples for each specific category of fresh meat considered.

3.3. *At border control posts*

(a) Sampling design:

When designing their sampling plan at border control posts, Member States shall take into account EFSA technical specifications on randomised sampling for harmonised monitoring of antimicrobial resistance in zoonotic and commensal bacteria⁽³⁾.

Member States shall ensure a proportionate stratified sampling of consignments and meat samples per border control post and country of origin with an even distribution over the monitoring year of the consignments of imported fresh meat sampled at border control posts level. All border control posts designated for fresh meat shall be included in the sampling plan. The consignments to be sampled on a given day shall be randomly selected and when sampling a consignment, samples shall be randomly taken. If a consignment is composed of different batches, the samples shall be taken from different batches. Samples shall not be pooled.

(b) Sample size:

Member States shall determine the appropriate number of samples they shall take per year from each fresh meat category referred to in points 1(a)(iv), 1(c)(v) and 1(d)(vi) based on the indicative sampling frequency rates set out in Table 1.

TABLE 1

Fresh meat subject to AMR testing at import: indicative sampling frequency rates

Type of fresh meat	Recommended annual sampling frequency rates of consignments arrived at the border control posts
Broiler meat	3 %
Turkey meat	15 %
Pig meat	10 %
Bovine meat	2 %

4. Antimicrobial susceptibility testing

4.1. *Number of isolates to be tested*

Member States shall test for antimicrobial susceptibility the following number of isolates annually and ensure that no more than one isolate per bacterial species/*Salmonella* serovar from the same epidemiological unit is tested per year:

For Salmonella spp:

— up to 170 isolates obtained from samples referred to in point 1(a)(i). Where Member States have a national annual production of less than 100 000 tonnes of broiler meat, they may decide to set an upper limit of 85 isolates instead of 170 isolates. The isolates shall be obtained from healthy animals. Where the number of isolates yearly available per animal population in a Member State is higher than the upper limit, a random selection of those isolates shall be performed in a way that ensures a geographical representativeness and, where possible, an even distribution of the date of sampling

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- over the year. When the number of isolates yearly available is lower than the upper limit, all of them shall be tested,
- at least 170 isolates obtained from samples referred to in point 1(a)(ii) or, for Member States making use of the derogation referred to in the second paragraph of point 3(1) (b), all isolates obtained from these samples. By way of derogation, where Member States have a national annual production of less than 100 000 tonnes of pig meat, they may decide to test a minimum of 85 isolates instead of 170 isolates,
- at least 170 isolates obtained from samples referred to in point 1(a)(iii) or, for Member States making use of the derogation referred to in the second paragraph of point 3(1) (b), all isolates obtained from these samples,
- all isolates obtained from samples referred to in point 1(a)(iv).

For *C. coli* and *C. jejuni*:

- at least 170 isolates of the nationally most prevalent species of *Campylobacter* (among *C. coli* and *C. jejuni*) obtained from samples referred to in point 1(b)(i) to (iii) or, for Member States making use of the derogation referred to in the second paragraph of point 3(1)(b), all isolates obtained from these samples. By way of derogation, where Member States have a national annual production of less than 100 000 tonnes of broiler meat, they may decide to test a minimum of 85 isolates instead of 170 isolates,
- up to 170 isolates of the nationally less prevalent species of *Campylobacter* (among *C. coli* and *C. jejuni*) identified while recovering the isolates of the most prevalent *Campylobacter* species obtained from samples referred to in point 1(b)(i) to (iii),
- at least 170 isolates of *C. coli* obtained from samples referred to in point 1(b)(iv) or, for Member States making use of the derogation referred to in the second paragraph of point 3(1)(b), all isolates obtained from these samples. By way of derogation, where Member States have a national annual production of less than 100 000 tonnes of pig meat, they may decide to test a minimum of 85 isolates instead of 170 isolates.

For indicator commensal E. coli:

- at least 170 isolates obtained from samples referred to in points 1(c)(i) to (iv). By way of derogation, where Member States have a national annual production of less than 100 000 tonnes of broiler meat, less than 100 000 tonnes of turkey meat or less than 100 000 tonnes of pig meat, they may decide to test a minimum of 85 isolates instead of 170 isolates for each specific animal population considered,
- all isolates obtained from samples referred to in point 1(c)(v).

For ESBL-, AmpC- and CP- producing E. coli:

- all isolates obtained from samples referred to in point 1(d).
- 4.2. Analytical methods for detection and antimicrobial susceptibility testing

Member States shall use the epidemiological cut-off values and the concentration ranges set out in Tables 2, 3 and 4 below to determine the antimicrobial susceptibility of *Salmonella* spp., *C. coli*, *C. jejuni*, indicator commensal *E. coli*, *E. faecalis* and *E. faecium*.

Any *E. coli* and *Salmonella* isolate tested in accordance with Table 2 showing resistance to cefotaxime or ceftazidime or meropenem shall be further tested with a second panel of antimicrobial substances in accordance with Table 5.

For the specific monitoring of ESBL-, AmpC- and/or CP-producing *E. coli*, Member States shall use the methods referred to in point 5.

The antimicrobial susceptibility testing shall be performed by the laboratories referred to in Article 3(2). The testing shall be performed by using the broth micro dilution method according to the reference method ISO 20776-1:2019.

TABLE 2

Panel of antimicrobial substances to be included in AMR monitoring, EUCAST thresholds for resistance and concentration ranges to be tested in *Salmonella* spp. and indicator commensal *E. coli* (First panel)

Antimicrobial Class of antimicrobial		Species	Interpretat of AMR (m	tive thresholds ng/L)	Range of concentrations
			ECOFF	Clinical breakpoint	(mg/L)(No of wells in brackets)
Amikacin	Aminoglycosic	Salmonella	> 4ª	> 16	4-128 (6)
		E. coli	> 8	> 16	
Ampicillin	Penicillin	Salmonella	> 8	> 8	1-32 (6)
		E. coli	> 8	> 8	
Azithromycin	Macrolide	Salmonella	NA	NA	2-64 (6)
		E. coli	NA	NA	
Cefotaxime	Cephalosporin	Salmonella	> 0,5	> 2	0,25-4 (5)
		E, coli	> 0,25	> 2	
Ceftazidime	Cephalosporin	Salmonella	> 2	> 4	0,25-8 (6)
		E, coli	> 0,5	> 4	
Chloramphenicol		Salmonella	> 16	> 8	8-64 (4)
		E, coli	> 16	> 8	
Ciprofloxacin	Fluoroquinolor	Salmonella	> 0,06	> 0,06	0,015-8 (10)
		E, coli	> 0,06	> 0,5	
Colistin	Polymyxin	Salmonella	NA	> 2	1-16 (5)
		E, coli	> 2	> 2	
Gentamicin	Aminoglycosic	Salmonella	> 2	> 4	0,5-16 (6)
		E, coli	> 2	> 4	
Meropenem	Meropenem Carbapenem	Salmonella	> 0,125	> 8	0,03-16 (10)
		E, coli	> 0,125	> 8	
Nalidixic acid	Quinolone	Salmonella	> 8	NA	4-64 (5)
	E, coli	> 8	NA		

a tentative EUCAST threshold

NA: not available.

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Sulfamethoxaz	Sulfamethoxazoffolate pathway antagonist	Salmonella	NA	NA	8-512 (7)
		E, coli	> 64	NA	
Tetracycline	Tetracycline	Salmonella	> 8	NA	2-32 (5)
		E, coli	> 8	NA	
Tigecycline	Glycylcycline	Salmonella	NA	NA	0,25-8 (6)
		E, coli	> 0,5	> 0,5	
Trimethoprim Folate	Salmonella	> 2	> 4	0,25-16 (7)	
	pathway antagonist	E, coli	> 2	> 4	

a tentative EUCAST threshold

NA: not available.

TABLE 3

Panel of antimicrobial substances to be included in AMR monitoring, EUCAST interpretative thresholds for resistance and concentration ranges to be tested in *C. jejuni* and *C. coli*

Antimicrobia	l Class of antimicrobial	Species	Interpretative thresholds of AMR (mg/L)		Range of concentrations
			ECOFF	Clinical breakpoint	(mg/L)(No of wells in brackets)
Chloramphenic	de lhenicol	C. jejuni	> 16	NA	2-64 (6)
		C. coli	> 16	NA	
Ciprofloxacin Fluoroquinolog	ı € . jejuni	> 0,5	> 0,5	0,12-32 (9)	
		C. coli	> 0,5	> 0,5	
Ertapenem Carbapenem	Carbapenem	C. jejuni	NA	NA	0,125-4 (6)
		C. coli	NA	NA	
Erythromycin	Macrolide	C. jejuni	> 4	> 4	1-512 (10)
		C. coli	> 8	> 8	
Gentamicin Aminoglycosio	Aminoglycosic	l€. jejuni	> 2	NA	0,25-16 (7)
		C. coli	> 2	NA	
Tetracycline Tetracycline	Tetracycline	C. jejuni	> 1	> 2	0,5-64 (8)
		C. coli	> 2	> 2	

NA: not available

TABLE 4

Panel of antimicrobial substances to be included in AMR monitoring, EUCAST thresholds for resistance and concentration ranges to be tested in *E. faecalis* and *E. faecium*

Antimicrobial Class of antimicrobial				tive thresholds ng/L)	Range of concentrations
		ECOFF	Clinical breakpoint	(mg/L)(No of wells in brackets)	
Ampicillin	Penicillin	E. faecalis	> 4	> 8	0,5-64 (8)
		E. faecium	> 4	> 8	
Chloramphenic	dhenicol	E. faecalis	> 32	NA	4-128 (6)
		E. faecium	> 32	NA	
Ciprofloxacin	Fluoroquinolor	Æ. faecalis	> 4	> 4	0,12-16 (8)
		E. faecium	> 4	> 4	
Daptomycin	Lipopeptide	E. faecalis	> 4	NA	0,25-32 (8)
		E. faecium	> 8	NA	
Erythromycin	Macrolide	E. faecalis	> 4	NA	1-128 (8)
		E. faecium	> 4	NA	
Gentamicin	Aminoglycosic	Æ. faecalis	> 64	NA	8-1 024 (8)
		E. faecium	> 32	NA	
Linezolid	Oxazolidinone	E. faecalis	> 4	> 4	0,5-64 (8)
		E. faecium	> 4	> 4	
Quinupristin/	Streptogramin	E. faecalis	NA	NA	0,5-64 (8)
Dalfopristin		E. faecium	NA	> 4	
Teicoplanin	Glycopeptide	E. faecalis	> 2	> 2	0,5-64 (8)
		E. faecium	> 2	> 2	
Tetracycline	Tetracycline	E. faecalis	> 4	NA	1-128 (8)
		E. faecium	> 4	NA	
Tigecycline	Glycylcycline	E. faecalis	> 0,25	> 0,25	0,03-4 (8)
		E. faecium	> 0,25	> 0,25	
Vancomycin	Glycopeptide	E. faecalis	> 4	> 4	1-128 (8)
		E. faecium	> 4	> 4	
NA: not available	ı	<u> </u>	- 1		

5. Specific monitoring of ESBL- or AmpC- or CP-producing E. coli

5.1. Methods for detection of presumptive ESBL- or AmpC- or CP-producing E. coli

For the purpose of estimating the proportion of samples containing presumptive ESBL- or AmpC- or CP-producing $E.\ coli$ among the caecal and fresh meat samples collected in accordance with point 1(d), the laboratories referred to in Article 3(2) shall use detection methods detailed in the protocols of the EURL for AMR⁽⁴⁾.

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All presumptive ESBL- or AmpC- or CP-producing *E. coli* isolates identified through the methods referred to in above shall be tested with the first panel and the second panel of antimicrobial substances in accordance with Table 2 and Table 5 respectively.

TABLE 5

Panel of antimicrobial substances, EUCAST epidemiological cut-off values (ECOFFs) and clinical resistance breakpoints and concentrations ranges to be used for testing only *Salmonella* spp. and *E. coli* isolates resistant to cefotaxime or ceftazidime or meropenem – (Second panel)

Antimicrobial Class of antimicrobial		Species	Interpretative thresholds of AMR (mg/L)		Range of concentrations
			ECOFF	Clinical breakpoint	(mg/L)(No of wells in brackets)
Cefepime	Cephalosporin	Salmonella	NA	> 4	0,06-32 (10)
		E. coli	> 0,125	> 4	
Cefotaxime	Cephalosporin	Salmonella	> 0,5	> 2	0,25-64 (9)
		E. coli	> 0,25	> 2	
Cefotaxime	Cephalosporin/	Salmonella	NA	NA	0,06-64 (11)
+ clavulanic acid	beta- lactamase inhibitor combination	E. coli	> 0,25	NA	
Cefoxitin	Cephamycin	Salmonella	> 8	NA	0,5-64 (8)
		E. coli	> 8	NA	
Ceftazidime	Cephalosporin	Salmonella	> 2	> 4	0,25-128 (10)
		E. coli	> 0,5	> 4	
Ceftazidime	Cephalosporin/	//Salmonella	NA	NA	0,125-128 (11)
+ clavulanic acid	beta- lactamase inhibitor combination	E. coli	> 0,5	NA	
Ertapenem	Carbapenem	Salmonella	NA	> 0,5	0,015-2 (8)
		E. coli	NA	> 0,5	
Imipenem	Carbapenem	Salmonella	> 1	> 4	0,12-16 (8)
		E. coli	> 0,5	> 4	
Meropenem Carbapenem	Carbapenem	Salmonella	> 0,125	> 8	0,03-16 (10)
		E. coli	> 0,125	> 8	
Temocillin	Penicillin	Salmonella	> NA	NA	0,5-128 (9)
		E. coli	> 16	NA	

5.2. Quantitative method to assess the proportion of ESBL- or AmpC-producing E. coli

Member States may decide to assess the proportion of ESBL- or AmpC-producing *E. coli* compared to the total *E. coli* isolates present in a sample. In this case they shall enumerate ESBL- or AmpC-producing *E. coli* and the total *E. coli* by using dilution methods and subsequent by plating onto selective media and non-selective media, according to the protocols of the EURL for AMR⁽⁵⁾.

6. **Alternative method**

Member States may decide to authorise the use of Whole Genome Sequencing ('WGS') as an alternative method to broth micro dilution using the testing panels of antimicrobial substances of Tables 2 and 5 when carrying out the specific monitoring of ESBL- or AmpC- or CP-producing *E. coli* as referred to in point 5. They may also authorise WGS as an alternative method to broth micro dilution using the testing panel of antimicrobial substances of Table 5 when further testing, in accordance with point 4.2, *E. coli* and *Salmonella* isolates showing resistance to cefotaxime or ceftazidime or meropenem.

Laboratories implementing WGS as an alternative method shall use the protocols of the EURL for AMR⁽⁶⁾.

7. Quality control, storage of the isolates and confirmatory testing

The Member States shall ensure participation of the laboratories referred to in Article 3(2) to a quality assurance system including proficiency testing set up at either national or Union level, to target species identification, sub-typing and antimicrobial susceptibility testing of the bacteria collected for the harmonised monitoring of AMR.

Resistant isolates shall be stored by the laboratories at a temperature of -80 °C for a minimum period of five years. Other temperatures of storage may be used provided that they ensure viability and absence of changes in strain properties.

When deemed scientifically relevant by EFSA and the EURL for AMR, the laboratories referred to in Article 3(2) shall send for a confirmatory testing to the EURL for AMR any isolate tested in accordance with points 4, 5 and 6.

- (1) https://www.efsa.europa.eu/it/efsajournal/pub/3686
- (2) See footnote 1.
- (3) See footnote 1.
- (4) https://www.eurl-ar.eu/protocols.aspx
- (5) https://www.eurl-ar.eu/protocols.aspx
- (6) https://www.eurl-ar.eu/protocols.aspx

Changes to legislation:

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