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COMMISSION IMPLEMENTING DECISION

of **XXX**

**on harmonized monitoring of antimicrobial resistance in zoonotic and commensal
bacteria**

(Text with EEA relevance)

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THE EUROPEAN COMMISSION,

Having regard to the Treaty on the Functioning of the European Union,

Having regard to Directive 2003/99/EC of the European Parliament and of the Council of 17 November 2003 on the monitoring of zoonoses and zoonotic agents, amending Council Decision 90/424/EEC and repealing Council Directive 92/117/EEC¹, and in particular Article 7(3) thereof,

Whereas:

- (1) Pursuant to Directive 2003/99/EC, Member States shall ensure that monitoring provides comparable data on the occurrence of antimicrobial resistance (AMR) in zoonotic agents and, in so far they present a threat to public health, other agents.
- (2) In the Communication from the Commission to the European Parliament and the Council on an five-year Action Plan against the Rising Threats from Antimicrobial Resistance², 12 concrete actions are proposed including a strengthen surveillance systems on AMR. During its meeting on 4 July 2012, the Council of the European Union adopted Conclusions on the Impact of Antimicrobial Resistance in the Human Health Sector and in the Veterinary Sector – a "One Health" Perspective³. The Council calls upon the Commission to follow up on its Communication through concrete initiatives to implement the 12 actions and, in particular to collaborate closely with the European Centre for Disease Prevention and Control (ECDC), the European Food Safety Authority (EFSA) and the European Medicines Agency (EMA) in strengthening the assessment and evaluation of the occurrence of AMR in humans, in animals and in food in the EU.
- (3) During its plenary sitting of 11 December 2012, the European Parliament adopted a Report on the Microbial Challenge – Rising Threats from Antimicrobial Resistance. The European Parliament welcomes the Commissions' five-year Action plan and considers that the measures recommended in the Action Plan need to be implemented as soon as possible. The European Parliament in particular calls on the Commission

¹ OJ L 325, 12.12.2003, p.31.

² COM (2011) 748

³ OJ C211, 18.7.2012, p.2.

and Member States to seek greater cooperation and coordination on early detection, alert and coordinated response procedures regarding pathogenic antimicrobial resistant bacteria in humans, animals, fish and foodstuffs in order to continuously monitor the extent and growth of AMR.

- (4) Under its Joint FAO/WHO Food Standards Programme, the Codex Alimentarius Commission adopted during its 34th Session in Geneva, the Guidelines for the Risk Analysis of Foodborne Antimicrobial Resistance⁴ highlighting AMR as a major global public health concern and a food safety issue. The use of antimicrobial agents in food producing animals/crops provides a potentially important risk factor for selection and dissemination of AMR microorganisms and determinants from animals/food crops to humans via the consumption of food.
- (5) The Codex Guidelines concluded *inter alia* that surveillance programmes on the prevalence of foodborne AMR provide information that is useful for all parts of the AMR risk analysis process. Methodology of surveillance programmes should be internationally harmonized to the extent possible. The use of standardized and validated antimicrobial susceptibility testing methods and harmonised interpretive criteria are essential to ensure that data are comparable.
- (6) In its Chapter 6.7 on “Harmonisation of National AMR Surveillance and Monitoring programmes”, the Terrestrial Animal Health Code of the World Animal Health Organisation (OIE)⁵ underlines the need for surveillance and monitoring of AMR to: assess and determine the trends and sources of antimicrobial resistance in bacteria, to detect the emergence of new antimicrobial resistance mechanisms, to provide the data necessary for conducting risk analyses as relevant to animal and human health, to provide a basis for policy recommendations for animal and human health and to provide information for evaluating antimicrobial prescribing practices and for prudent use recommendations.
- (7) On 9 July 2008, the European Food Safety Authority (EFSA) adopted a Scientific Opinion on Foodborne Antimicrobial Resistance as a Biological Hazard⁶. On 28 October 2009, ECDC, EFSA, EMA and the European Commission’s Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR) published a joint Scientific Opinion on Antimicrobial Resistance (AMR) focused on infections transmitted to humans from animals and food (zoonoses)⁷. On 5 March 2009, the EFSA adopted a Scientific Opinion on the Assessment of the Public Health Significance of Meticillin Resistant *Staphylococcus aureus* (MRSA)⁸. On 7 July 2011, the EFSA adopted a Scientific Opinion on the Public Health Risks of Bacterial Strains producing ESBL [Extended-Spectrum β -Lactamases] and/or AmpC [AmpC β -Lactamases] in food and food-producing animals⁹. On 3 October 2011, EFSA adopted a Technical Report on Approaches to Risk Assessment in the area of Antimicrobial Resistance, with an Emphasis on Commensal Microorganisms¹⁰. The main conclusion of all these opinions and reports is that, in view of the increasing public health concern

⁴ CAC/GL 77-2011

⁵ <http://www.oie.int>

⁶ *The EFSA Journal* (2008) 765, 1-87.

⁷ *EFSA Journal* 2009; 7(11):1372.

⁸ *The EFSA Journal* (2009) 993, 1-73.

⁹ *EFSA Journal* 2011;9(8):2322.

¹⁰ *EFSA Journal* 2011;9(10):196.

on AMR, the use of harmonised methods and epidemiological cut-off values is necessary to ensure the comparability of data over time at country level, and also to facilitate the comparison of the occurrence of AMR between Member States.

- (8) On 14 June 2012, EFSA published a Scientific Report on Technical Specifications on the Harmonised Monitoring and Reporting of Antimicrobial Resistance in *Salmonella*, *Campylobacter* and indicator commensal *Escherichia coli* and *Enterococcus* spp. bacteria transmitted through Food¹¹. On 5 October 2012, the EFSA published a Scientific Report on Technical Specifications on the Harmonised Monitoring and Reporting of Antimicrobial Resistance in Methicillin-Resistant *Staphylococcus aureus* (MRSA) in Food-producing Animals and Food¹². The scientific reports provide recommendations for detailed rules on a harmonised monitoring and reporting on the prevalence of resistant microorganisms in food-producing animals and food, in particular as regards the microorganisms to be included, the origin of the isolates of the microorganisms, the number of isolates to be tested, the antimicrobial susceptibility tests to be used, the specific monitoring of MRSA and ESBL or AmpC - producing bacteria, and the collection and reporting of the data. The involvement of ECDC in this work will ensure the comparison between the data of the food producing animals and food sector and the data of the human sector.
- (9) When defining the combinations of bacterial species, food animal species and/or food products to be included in the harmonized monitoring AMR, it is important to prioritize the most relevant from a public health perspective. In order to minimize the burden, the monitoring will derive as much as possible from biological samples or isolates collected in the context of already established control programs. The monitoring of AMR in *Salmonella* will be focused on isolates deriving from the *Salmonella* national control programs and from the testing and verification set up by the competent authority in accordance with the legislation on microbiological criteria for foodstuffs.
- (10) Commission Decision 2007/407/EC of 12 June 2007 on a harmonised monitoring of AMR in *Salmonella* in poultry and pigs¹³ lays down detailed rules for the monitoring of AMR to be carried out by Member States, covering *Salmonella* spp in fowl, turkeys and slaughter pigs for a period of 5 years, from 2007 until 2012. Such harmonised monitoring should be continued to follow the evolution of trends and be extended to AMR in other pathogens and commensals in line with the increasing public health concern on the role of these microorganisms in the overall risk of AMR indicated by several scientific opinions. Monitoring according to Article 7 of Directive 2003/99/EC should therefore be based on the recommendations in the EFSA reports with technical specifications on the harmonised monitoring and reporting of AMR without prejudice to further implementing rules in the future.
- (11) The measures provided for in this Decision are in accordance with the opinion of the Standing Committee on the Food Chain and Animal Health,

¹¹ EFSA Journal 2012;10(6):2742

¹² EFSA Journal 2012;10(10):2897

¹³ OJ L 153, 14.6.2007, p. 26.

HAS ADOPTED THIS DECISION:

Article 1

Subject matter and scope

This Decision lays down detailed rules for harmonized monitoring and reporting of AMR in accordance with Article 7(3) and Annex II(B) of Directive 2003/99/EC to be carried out by the Member States. It shall cover isolates of *Salmonella* spp. *Campylobacter jejuni*, *Campylobacter coli*, indicator commensal *Escherichia coli*, *Enterococcus faecalis* and *Enterococcus faecium* from samples obtained from certain food-producing animals and in certain food thereof. This Decision lays down specific requirements for a harmonized monitoring of Extended-Spectrum β -Lactamases (ESBL) or AmpC β -Lactamases (AmpC-) or carbapenemase producing bacteria in certain food producing animals and in certain food thereof .

Article 2

Collection and analyses of isolates

Member States shall collect representative isolates of *Salmonella* spp., *Campylobacter jejuni*, indicator commensal *E. coli*, and ESBL- or AmpC- or carbapenemase producing *Salmonella* and *E. coli*, in accordance with the technical specifications set out in the Annex.

Member States may also decide to collect representative isolates of *Campylobacter coli*, indicator commensal *Enterococcus faecalis* and *Enterococcus faecium*. When doing it, it shall be done in accordance with the specifications set out in the Annex.

In addition, if due to the low bacterial prevalence and/or low number of epidemiological units in a Member State the minimum number of *Salmonella* isolates collected in official sampling is not sufficient to achieve the minimal required number of isolates to be tested for antimicrobial susceptibility, isolates obtained by food business operators in accordance with point 2.1.3, 2.1.4 and 2.1.5 in Chapter 2 to Annex I of Regulation (EC) No 2073/2005 of 15 November 2005 on microbiological criteria for foodstuffs¹⁴ and article 5 of 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 agents¹⁵ may also be used by the competent authority for antimicrobial susceptibility testing for *Salmonella* according with the technical specifications set out in the Annex. Food business operators shall ensure that isolates are available for antimicrobial susceptibility testing by the Competent Authority at the request of the Competent Authority. When using this option, the competent authorities shall ensure the representativeness of the isolates.

National reference laboratories for antimicrobial resistance shall perform the antimicrobial susceptibility testing of the isolates and the specific monitoring of ESBL- or AmpC- or

¹⁴ OJ L 338, 22.12.2005, p. 1

¹⁵ OJ L 325, 12.12.2003, p. 1.

carbapenemase producing *Salmonella* and *E. coli*. The competent authority may designate other laboratories than the national reference laboratories in accordance with article 12 of Regulation (EC) No 882/2004 to perform the analysis.

Article 3

Confidentiality of the data

National isolated-based quantitative antimicrobial resistance data and results of the analyses shall be made available publicly in a form that ensures confidentiality.

Article 4

Review

This Decision shall be reviewed taking into account progress in science, technology and methodology, information from risk assessment and scientific bodies, the results and trends of the harmonized monitoring and other relevant information.

Article 5

Repeal

Commission Decision 2007/407/EC of 12 June 2007 on a harmonised monitoring of AMR in *Salmonella* in poultry and pigs is hereby repealed.

Article 6

Application

This Decision shall enter into force on the 20st day following that of its publication in the *Official Journal of the European Union*.

It shall apply from 1 January 2014.

This Decision is addressed to the Member States.

Done at Brussels,

For the Commission

(PE/PO)

The President

[...]

ANNEX

TECHNICAL SPECIFICATIONS REFERRED TO IN ARTICLE 2

PART A

SAMPLING FRAME AND ANALYSIS OF THE ISOLATES

1. Origin of isolates

Representative isolates shall be collected for monitoring AMR from at least each of the following domestically produced animal population or food category specified below:

- (a) *Salmonella* isolates shall be collected from:
 - (i) each population of laying hens, broilers, and fattening turkeys through national control programmes, set up in accordance with Article 5 of Regulation (EC) No 2160/2003;
 - (ii) carcasses of both broilers and turkeys sampled for the testing and verification, set up in accordance with point 2.1.5 in Chapter 2 to Annex I of Regulation (EC) No 2073/2005;
 - (iii) carcasses of pigs sampled for the testing and verification, set up in accordance with point 2.1.4 in Chapter 2 to Annex I of Regulation (EC) No 2073/2005;
 - (iv) carcasses of calves under 1 year of age when the production of calf meat in the Member State is more than 10.000 tonnes slaughtered per year sampled for the testing and verification, set up in accordance with point 2.1.3 in Chapter 2 to Annex I of Regulation (EC) No 2073/2005.
- (b) *Campylobacter jejuni* isolates shall be collected from
 - caecal samples gathered at slaughter from broilers and from fattening turkeys when the production of turkey meat in the Member State is more than 10.000 tonnes slaughtered per year.
- (c) Indicator commensal *Escherichia coli* isolates shall be collected from:
 - (i) caecal samples gathered at slaughter from broilers and from fattening turkeys when the production of turkey meat in a Member State is more than 10.000 tonnes slaughtered per year;
 - (ii) caecal samples gathered at slaughter from fattening pigs and calves under 1 year of age when the production of calf meat in the Member State is more than 10.000 tonnes slaughtered per year.

- (d) Specific monitoring of ESBL- or AmpC- or carbapenemase producing *E. coli* shall be performed in:
- (i) caecal samples gathered at slaughter from broilers and from fattening turkeys when the production of turkey meat in a Member State is more than 10.000 tonnes slaughtered per year;
 - (ii) caecal samples gathered at slaughter from fattening pigs and calves under 1 year of age when the production of calf meat in the Member State is more than 10.000 tonnes slaughtered per year;
 - (iii) fresh meat of broilers, pork and beef samples gathered at retail.
- (e) If a Member State decides to test *Enterococcus faecalis* or *Enterococcus faecium* in accordance with article 2, isolates shall be collected from:
- (i) caecal samples gathered at slaughter from broilers and from fattening turkeys when the production of turkey meat in a Member State is more than 10.000 tonnes slaughtered per year;
 - (ii) caecal samples gathered at slaughter from fattening pigs and calves under 1 year of age when the production of calf meat in the Member State is more than 10.000 tonnes slaughtered per year.
- (f) If a Member State decides to test *Campylobacter coli* in accordance with article 2, isolates shall be collected from:
- (i) caecal samples gathered at slaughter from broilers;
 - (ii) caecal samples gathered at slaughter from fattening pigs.

Isolates obtained by the competent authority from an origin other than the origin mentioned in points (a) to (f), including imported animals or food and isolates obtained at other points of the food chain may be tested for AMR on a voluntary basis and reported separately. However, when carrying out such testing, the specific technical requirements of point 3 to 5 of this Annex shall apply.

2. Sampling design and number of isolates to be tested

2.1 Sampling frequency

The antimicrobial susceptibility testing of each combination of bacterial species and type of sample of food producing animal population or food thereof listed in point 1 of this Annex and the specific monitoring of ESBL- or AmpC- or carbapenemase producing *Salmonella* and *E. coli* in accordance with point 4 of this Annex shall be carried out every two years according to the following rotation system:

- a) The antimicrobial susceptibility testing of each combination of bacterial species and type of sample and the specific monitoring of ESBL- or AmpC- or carbapenemase producing *Salmonella* and *E. coli* shall be carried out on laying hens, broilers and turkeys and food thereof in 2014, 2016, 2018 and 2020. However, the specific monitoring of ESBL- or AmpC- or carbapenemase producing indicator commensal *E. coli* in accordance with point 4.1 of this annex will not be mandatory in 2014.
- b) The antimicrobial susceptibility testing of each combination of bacterial species and type of sample and the specific monitoring of ESBL- or AmpC- or carbapenemase producing *Salmonella* and *E. coli* shall be carried out on pigs and calves and food thereof in 2015, 2017 and 2019.

2.2 Sample size

Member States shall test 170 isolates for antimicrobial susceptibility testing for each combination of bacterial species and type of sample of food producing animal population or food thereof listed in point 1(a), 1(b), 1(c), 1(e) and 1(f) of this Annex.

In those Member States where, in any given year, a higher number of isolates for some of the combinations of bacterial species and sample type of food producing animal populations or food thereof is available, all isolates or a representative random selection equal to or larger than 170, shall be included in the susceptibility testing.

In those Member States where due to the low prevalence and/or low number of epidemiological units, in any given year, the number of 170 isolates for some of the combinations of bacterial species and sample type of food producing animal population or food thereof, cannot be achieved, all available isolates at the end of the monitoring period shall be included in the antimicrobial susceptibility testing.

For the specific monitoring of ESBL or AmpC- or carbapenemase producing indicator commensal *E. coli* described in point 4.1, Member States shall analyse 300 samples¹⁶ of each of the food producing animal populations or food thereof, listed in point 1(d) of this Annex,

The results of additional AMR monitoring shall not influence the number of 170 isolates for each bacterial species/animal population or bacterial species/food combination to be tested according to the harmonized monitoring in point 1 of this Annex.

2.3 Sampling design

Isolates which are tested for antimicrobial susceptibility shall derive from active monitoring programmes, based on randomised sampling design. The bacterial isolates should originate from healthy animals sampled from randomly selected epidemiological units or randomly selected within the slaughterhouses. If diseased animals are sampled, these susceptibility results should be reported separately.

The competent authority shall ensure the randomization of the scheme and its correct implementation.

In the case of sampling at slaughterhouses, it is recommended that slaughterhouses processing at least 60 % of the specific domestic animal population (starting with the slaughterhouses of largest throughput) are eligible for sampling.

¹⁶ Based on 95% confidence level to detect an estimated prevalence of 1 %

Not more than one isolate per bacterial species from the same epidemiological unit per year shall be included in the monitoring. The epidemiological unit for laying hens, broilers, and turkeys is the flock. For pigs and calves, the epidemiological unit is the holding.

2.3.1. Representative sampling of caecal samples at slaughter

The random sampling plan should be typically stratified per slaughterhouse by allocating the number of samples collected per slaughterhouse proportionally to the annual throughput of the slaughterhouse. The collected samples should be evenly distributed over optimally each month of the year to enable the different seasons to be covered. Only one representative sample of caecal content per epidemiological unit, derived either from a unique carcass or from a number of carcasses, is gathered to account for clustering. The sampling must otherwise be based on a random selection regarding sampling days each month and which batches are to be sampled on a selected sampling day.

The number of biological samples to be collected is determined in order to achieve 170 isolates by accounting for the prevalence of the bacteria species monitored.

2.3.2. Collection of representative Salmonella isolates recovered in the framework of the National Control Programme of Salmonella in relevant animal populations and recovered in the framework through testing and verification, set up in accordance with point 2.1.3, 2.1.4, 2.1.5 in Chapter 2 to Annex I of Regulation (EC) No 2073/2005

Susceptibility testing should be done for no more than one isolate per *Salmonella* serovar from the same epidemiological unit per year.

In the case of a number of *Salmonella* isolates yearly available per animal population higher than 170 in the Member State, a random selection of at least 170 isolates should be performed from the collection of yearly available isolates in the Member State, in a way that ensures geographical representativeness and even distribution of the date of sampling over the year. Conversely, in the case of low prevalence, all the *Salmonella* isolates available should be tested for susceptibility.

3. Antimicrobials for susceptibility testing, epidemiological cut off values and concentration ranges to be used for antimicrobial susceptibility testing of the isolates

Member States shall test at least the antimicrobials and interpret the results using the epidemiological cut-off values and at least the concentration range that are specified in the relevant Tables 1, 2 and 3, to determine the susceptibility of *Salmonella*, *C. coli*, *C. jejuni*, indicator commensal *E. coli*, *E. faecalis* and *E. faecium*.

Dilution methods shall be performed according to the methods described by the European Committee on Antimicrobial Susceptibility Testing (EUCAST) and the Clinical and Laboratory Standards Institute (CLSI), accepted as the international reference method (ISO standard 20776-1:2006).

Table 1: Panel of antimicrobial substances to be included in AMR monitoring, EUCAST thresholds for resistance and concentration ranges to be tested in *Salmonella* and indicator commensal *E. coli* (First panel)

Antimicrobial	Species	Interpretative thresholds of AMR (mg/L)		Range of concentrations (mg/L) (No of wells in brackets)
		ECOFF ^a	Clinical resistance breakpoint ^b	
Ampicillin	<i>Salmonella</i>	>8	>8	1-64 (7)
	<i>E. coli</i>	>8	>8	
Cefotaxime	<i>Salmonella</i>	>0.5	>2	0.03-4 (8)
	<i>E. coli</i>	>0.25	>2	
Ceftazidime	<i>Salmonella</i>	>2	>4	0.06-8 (8)
	<i>E. coli</i>	>0.5	>4	
Meropenem	<i>Salmonella</i>	>0.125	>8	0.03-16 (10)
	<i>E. coli</i>	>0.125	>8	
Nalidixic acid	<i>Salmonella</i>	>16	NA	4-128 (6)
	<i>E. coli</i>	>16	NA	
Ciprofloxacin	<i>Salmonella</i>	>0.064	>1	0.008-8 (11)
	<i>E. coli</i>	>0.064	>1	
Tetracycline	<i>Salmonella</i>	>8	NA	2-64 (6)
	<i>E. coli</i>	>8	NA	
Colistin	<i>Salmonella</i>	>2	>2	0.5-16 (6)
	<i>E. coli</i>	>2	>2	
Gentamicin	<i>Salmonella</i>	>2	>4	0.5-32 (7)
	<i>E. coli</i>	>2	>4	
Trimethoprim	<i>Salmonella</i>	>2	>4	0.25-32 (8)
	<i>E. coli</i>	>2	>4	
Sulfamethoxazole	<i>Salmonella</i>	NA	NA	8-1024 (8)
	<i>E. coli</i>	>64	NA	
Chloramphenicol	<i>Salmonella</i>	>16	>8	8-256 (6)
	<i>E. coli</i>	>16	>8	

^a: EUCAST epidemiological cut-off values

^b EUCAST clinical breakpoints

NA: not available

Table 2: 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*

Antimicrobial	Species	Interpretative thresholds of AMR (mg/L)		Range of concentrations (mg/L) (No of wells in brackets)
		ECOFF ^a	Clinical resistance	

			breakpoint ^b	
Erythromycin	<i>C. jejuni</i>	>4	>4	1-128 (8)
	<i>C. coli</i>	>8	>8	
Ciprofloxacin	<i>C. jejuni</i>	>0.5	>0.5	0.12-16 (8)
	<i>C. coli</i>	>0.5	>0.5	
Tetracycline	<i>C. jejuni</i>	>1	>2	0.5-64 (8)
	<i>C. coli</i>	>2	>2	
Gentamicin	<i>C. jejuni</i>	>2	NA	0.12-16 (8)
	<i>C. coli</i>	>2	NA	
Nalidixic acid	<i>C. jejuni</i>	>16	NA	1-64 (7)
	<i>C. coli</i>	>16	NA	

^a: EUCAST epidemiological cut-off values

^b EUCAST clinical breakpoints

NA: not available

Table 3: panel of antimicrobial substances to be included in AMR monitoring, EUCAST thresholds and concentration ranges to be tested in *E. faecalis* and *E. faecium*

Antimicrobial	Species	Interpretative thresholds of AMR (mg/L)		Range of concentrations (mg/L) (No of wells in brackets)
		ECOFF ^a	Clinical resistance breakpoint ^b	
Gentamicin	<i>E. faecalis</i>	>32	NA	8-1024 (8)
	<i>E. faecium</i>	>32	NA	
Chloramphenicol	<i>E. faecalis</i>	>32	NA	4-128 (6)
	<i>E. faecium</i>	>32	NA	
Ampicillin	<i>E. faecalis</i>	>4	>8	0.5-64 (8)
	<i>E. faecium</i>	>4	>8	
Vancomycin	<i>E. faecalis</i>	>4	>4	1-128 (8)
	<i>E. faecium</i>	>4	>4	
Teicoplanin	<i>E. faecalis</i>	>2	>2	0.5-64 (8)
	<i>E. faecium</i>	>2	>2	
Erythromycin	<i>E. faecalis</i>	>4	NA	1-128 (8)
	<i>E. faecium</i>	>4	NA	
Quinupristin/ Dalfopristin	<i>E. faecalis</i>	NA	NA	0.5-64 (8)
	<i>E. faecium</i>	>1	>4	
Tetracycline	<i>E. faecalis</i>	>4	NA	1-128 (8)
	<i>E. faecium</i>	>4	NA	
Tigecycline	<i>E. faecalis</i>	>0.25	>0.5	0.03-4 (8)
	<i>E. faecium</i>	>0.25	>0.5	
Linezolid	<i>E. faecalis</i>	>4	>4	0.5-64 (8)
	<i>E. faecium</i>	>4	>4	
Daptomycin	<i>E. faecalis</i>	>4	NA	0.25-32 (8)
	<i>E. faecium</i>	>4	NA	

^a: EUCAST epidemiological cut-off values

^b EUCAST clinical breakpoints

NA: not available

4. Specific monitoring of ESBL- or AmpC- or carbapenemase producing *Salmonella* and *E. coli*

4.1. Detection method of ESBL- or AmpC- or carbapenemase producing *E. coli* in broilers, fattening turkeys, fattening pigs, calves under one year, and meat thereof

For the purpose of estimating the proportion of samples containing ESBL- or AmpC- or carbapenemase producing *E. coli* amongst the caecal samples collected from broilers, fattening turkeys, fattening pigs, calves under 1 year of age, fresh poultry meat, pork and beef, the following method shall apply.

For ESBL- or AmpC-producing *E. coli* the method shall start by a pre-enrichment step, using a selective enrichment broth containing a cephalosporin (1 mg/L of ceftriaxone). The enrichment shall be followed by inoculation on McConkey agar containing 1 mg/L of ceftriaxone. The microbial species *E. coli* shall be identified using an appropriated method.

The Member State may decide, based on the epidemiological circumstances, to use in parallel additional selective plate that inhibits for growth of AmpC to facilitate the specific detection of ESBLs. When using this possibility, the results of the additional selective plate that inhibits for growth of AmpC shall be reported separately.

Additionally, Member States may decide to detect carbapenemase-producing micro-organisms by using selective pre-enrichment and subsequent selective plating in carbapenem-containing media, according to the most recent version of the detailed protocol for standardization of the European Union Reference Laboratory AMR.¹⁷

The obtained presumptive ESBL- or AmpC- or carbapenemase producing *E. coli* shall be tested on the first panel of antimicrobials according to Table 1 of Chapter 3 of this annex and further submitted to extended susceptibility testing as described under point 4.2 of this chapter if they are resistant to cefotaxime or ceftazidime or meropenem.

4.2. Method for further characterisation and classification of *Salmonella* and *E. coli* isolates showing resistance to third-generation cephalosporins or meropenem

All those randomly selected isolates of *Salmonella* and *E. coli* that after testing with the first panel of antimicrobials (Table 1 of Chapter 3 of this annex), are resistant to cefotaxime or ceftazidime or meropenem, shall be further tested with a second of antimicrobial substances according to Table 4 , including examination for clavulanate synergy and/or carbapenemase-production confirmatory test, according to the most recent version of the detailed protocol of the European Union Reference Laboratory AMR¹⁸.

¹⁷ www.crl-ar.eu

¹⁸ www.crl-ar.eu

Table 4: panel of antimicrobial substances, EUCAST epidemiological cut-off values (ECOFFs) and clinical resistance breakpoints and concentrations ranges to be used for testing only *Salmonella* and indicator commensal *E. coli* isolates resistant to cefotaxime or ceftazidime or meropenem – (Second panel)

Antimicrobial	Species	Interpretative thresholds of AMR (mg/L)		Range of concentrations (mg/L) (No of wells in brackets)
		ECOFF ^a	Clinical resistance breakpoint ^b	
Cefoxitin	<i>Salmonella</i>	>8	NA	0.5-64 (8)
	<i>E.coli</i>	>8	NA	
Cefepime	<i>Salmonella</i>	NA	NA	0.06-32 (10)
	<i>E.coli</i>	>0.125	>4	
Cefotaxime + clavulanic acid*	<i>Salmonella</i>	NA**	NA**	0.06-64 (11)
	<i>E.coli</i>	NA**	NA**	
Ceftazidime + clavulanic acid*	<i>Salmonella</i>	NA**	NA**	0.125-128 (11)
	<i>E.coli</i>	NA**	NA**	
Tigecycline	<i>Salmonella</i>	>1***	>2***	0.06-8 (8)
	<i>E.coli</i>	>1	>2	
Florfenicol	<i>Salmonella</i>	>16	NA	2-256 (8)
	<i>E.coli</i>	>16	NA	
Imipenem	<i>Salmonella</i>	>1	>8	0.12-16 (8)
	<i>E.coli</i>	>0.5	>8	
Ertapenem	<i>Salmonella</i>	>0.06	>1	0.015-2 (8)
	<i>E.coli</i>	>0.06	>1	
Azithromycin	<i>Salmonella</i>	NA	NA	1-64 (7)
	<i>E.coli</i>	NA	NA	

^a: EUCAST epidemiological cut-off values

^b EUCAST clinical breakpoints

NA: not available

*4 mg/L clavulanic acid

** The values have to be compared to the values from Panel 1 (Table 1) and interpreted according to CLSI guidelines regarding synergy testing.

*** Data from EUCAST available only for *Salmonella* Enteritidis, Typhimurium, Typhi and Paratyphi

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

Member States may also decide to characterise the importance of ESBL-producing *E. coli* within the whole *E. coli* population, and therefore, determine the proportion of *E. coli* which are ESBL- producing *E. coli*, in particular in those Member States which have detected a high prevalence of ESBL- producing *E. coli* by the detection method.

When doing it, it shall be done by enumerating ESBL/AmpC-producing *E. coli* and total *E. coli* present in a sample using dilution methods and subsequent plating onto selective media and non-selective media, according to the most recent version of the detailed protocol of the European Union Reference Laboratory AMR¹⁹.

5. Quality control and storage of the isolates

The laboratories designated by the competent authority to perform the antimicrobial susceptibility testing of the isolates included in the harmonized monitoring program, shall be involved in a quality assurance system including proficiency test set up either at national or EU level, in identification, typing and susceptibility testing of the bacteria targeted by the harmonised monitoring of AMR.

Isolates may be stored by the national reference laboratories for Antimicrobial Resistance at a temperature of -80°C for a minimum period of 5 years. Other methods of storage may alternatively be used provided that they ensure viability and absence of changes in strain properties.

¹⁹

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PART B

REPORTING

6. General provisions

The results of the AMR monitoring shall be assessed and reported, in accordance with Article 9 of Directive 2003/99/EC, in the yearly report on trends and sources of zoonoses, zoonotic agents and antimicrobial resistance.

If AMR monitoring is performed by the competent authority from isolates obtained by the competent authority at other stages of the food chain but using the methodology described in this Decision, the results of this additional AMR monitoring maybe be separately reported but this will not influence the number of isolates to be tested according to the harmonized monitoring.

7. Information to be included for each individual sample

Without prejudice to the provisions of Annex IV of Directive 2003/99/EC, reports shall be made including at least the following information for each individual isolate, considering separately each bacterial species/animal population or bacterial species/food combination referred to in point 1 of this Annex.

Member States shall submit the results of the investigation in the form of raw isolate-based data using a data dictionary and an electronic collection forms provided by EFSA²⁰.

7.1. Overall description of the implementation of the antimicrobial resistance monitoring programme

- Description of sampling designs, stratification and randomisation procedures per animal populations and food categories.

7.2. General information

- Identifier/code of the isolate (unique identifier at the reporting Member State level)
- Bacterial species (*Salmonella*, *C. jejuni*, *C. coli*, *E. coli*, *E. faecium*, *E. faecalis*)
- Serotype (for *Salmonella*)
- Phage type of *Salmonella* Enteritidis and *Salmonella* Typhimurium (optional)

7.3. Specific information with regard to the sampling

- Food-producing animal population/food category (e.g. laying hens, broilers, calves under 1 year of age, broiler meat, turkey meat, pig meat, etc.)

²⁰ www.efsa.europa.eu

- Stage of sampling (e.g. on farm, at slaughterhouse, at retail, etc.)
- Type of sample (e.g. caecal content, boot swabs, nasal swab, neck skin, carcase swab etc.)
- Sampler (e.g. Industry sampling, official sampling, etc.)
- The sampling strategy (e.g. objective sampling, census sampling, etc.)
- Date of sampling
- Date of isolation

7.4. Specific information with regard to antimicrobial resistance testing

- Identifier/code of the isolate given by the laboratory performing the susceptibility testing of the isolate
- Date of susceptibility testing
- Antimicrobial substance

7.5. Specific information with regard to dilution method results

- Minimal Inhibitory Concentration (MIC) value (in mg/L)

7.6. Synergy testing results

- Synergy testing with clavulanic acid for ceftazidime
- Synergy testing with clavulanic acid for cefotaxime