Technical specifications on a randomisation of sampling for the purpose of antimicrobial resistance monitoring from food-producing animals and food as from 2021

European Food Safety Authority (EFSA)

Abstract
To monitor antimicrobial resistance in zoonotic and indicator bacteria from food-producing animal populations and meat thereof under Decision 2020/1729, a guidance for randomised sampling procedures is provided. Prospective and retrospective sampling plans for samples and isolates are addressed. The former involves collecting sufficient numbers of representative animal and food samples from which recovered isolates are tested for antimicrobial susceptibility; the latter involves selecting randomly *Salmonella* isolates from collections constituted within the framework of the national control programmes in poultry flocks. A generic proportionate stratified sampling process and numerical illustrations of proportional allocation are provided. Stratified sampling of *Salmonella* isolates from poultry primary productions is performed with proportional allocation to the size of the isolate collections available in official laboratories. An alternative approach would be a simple random sampling within the sampling frame of flocks positive for *Salmonella*. Stratified sampling of caecal samples, accounting for at least 60% of the domestic production of food-producing animal populations monitored, with proportionate allocation to the slaughterhouse production, allows for the collection of representative isolates of *Campylobacter* and indicator *E. coli* and enterococci in various animal populations. Sampling of different chilled fresh meat categories is performed at retail outlets serving the final consumer, with proportional allocation of the number of samples to the population of geographical areas accounting for at least 80% of the national population, to test for the presence of ESBL-/AmpC-/carbapenemase-producing *E. coli*. Stratified sampling of imported fresh meat is performed at border control posts, with proportional allocation to the number of consignments and origin to test *Salmonella* and indicator *E. coli* for antimicrobial susceptibility, and to test for the presence of ESBL-/AmpC-/carbapenemase-producing *E. coli*. The corresponding sampling design is based on the reliable existing TRACES statistics, and the effect of the UK leaving the EU cannot be considered at this stage because of the major uncertainties still associated with it. These technical specifications should be updated as needed based on the first monitoring campaigns and trends in AMR.

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**Keywords:** monitoring, antimicrobial resistance monitoring, randomised sampling, food-producing animals, food

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Summary

In accordance with Directive 2003/99/EC on monitoring of zoonoses and zoonotic agents, Member States (MSs) must ensure that monitoring provides comparable data on the occurrence of antimicrobial resistance (AMR) in zoonotic agents. The Commission Implementing Decision 2020/1729 lays down specific technical requirements for AMR testing and reporting in representative isolates deriving from randomised sampling of broilers, laying hens, fattening turkeys, fattening pigs and calves, performed at farm and/or at slaughter, and of fresh meat from broilers, turkeys, pigs and bovine animals performed at retail and at border control posts. The European Food Safety Authority (EFSA) received a mandate from the European Commission to provide recommendations on harmonised randomisation procedures for AMR monitoring. This scientific report provides a short rationale and harmonised functional procedures for randomised sampling of animal and meat samples at different stages of the food chain, yielding representative and comparable data. A key principle considered in relation to sampling design is to reinforce harmonised functional procedures for randomised sampling of animal and meat samples at different stages of the food chain, yielding representative and comparable data.

Both collection strategies, a prospective and a retrospective sampling plan, of samples and isolates, respectively, are retained. The former involves collecting sufficient numbers of representative animal and fresh meat samples from which recovered isolates are tested for antimicrobial susceptibility; the latter involves selecting randomly Salmonella isolates from collections constituted within the framework of the national control programmes of Salmonella in flocks of laying hens, broilers and fattening turkeys. A generic proportionate stratified sampling process is proposed for the different sampling plans and numerical illustrations of proportional allocation are also provided. The epidemiological units are flocks of poultry, slaughter batches of fattening pigs and bovine animals under 1 year, and lot of meat at retail level and consignment of meat at border control posts.

Stratified sampling of Salmonella isolates recovered from broiler, laying hen and fattening turkey primary productions, and available in the collection of the laboratories involved in the Salmonella national control programmes, with proportional allocation to the size of the collection of isolates recovered from the production, is proposed. An alternative approach is to perform a simple random sampling within the sampling frame of flocks positive for Salmonella in those MS where a database records flocks tested positive for Salmonella. One Salmonella isolate per serovar and epidemiological unit should be retained for susceptibility testing.

Stratified sampling of caecal content samples (single or pooled) in the slaughterhouses, accounting for at least 60% of the national domestic production of the food-producing animal populations monitored, with proportionate allocation to the slaughterhouse production, allows for the collection of representative isolates of Campylobacter jejuni, Campylobacter coli, indicator E. coli, enterococci and the assessment of the prevalence of ESBL-/AmpC-/carbapenemase-producing E. coli from the populations of broilers, fattening turkeys, fattening pigs and bovine animals under 1 year of age, domestically produced. Definitions of ‘domestically produced’ animals are provided for the sake of harmonisation.

Stratified sampling of different imported fresh meat categories is performed at border control posts, with proportional allocation of the number of samples to the number of consignments received per border control post and country of origin, to test Salmonella and indicator E. coli for antimicrobial susceptibility, and to test for the presence of ESBL-/AmpC-/carbapenemase-producing E. coli. Every EU MS and every EFTA country concerned (Switzerland, Iceland and Norway) receiving consignments of fresh meat of broilers, turkeys, pigs and bovine animals from third countries to EU/EEA/EFTA is included in the sampling frame. All border control posts designated for fresh meat shall be included in the sampling plan. The sampling design proposed is based on the reliable existing TRACES statistics, and the effect of the UK leaving the EU in 2021 cannot be considered at this stage because of the major uncertainties still associated with it, regarding probable changes in both trading arrangements and trade flows. These technical specifications highlight those uncertainties. As the sampling fractions and number of samples to be taken per consignment are indicative, MSs will also have the possibility to adapt them to their particular situation, in particular, regarding pig meat and turkey meat. If MSs have already detailed data available, MSs can consider them when designing the sampling plan. The monitoring of AMR in imported meat is introduced in 2021, and the year 2021 will allow to gain experience and knowledge on this topic, as well as relevant TRACES statistics.

Stratified sampling of different chilled fresh meat categories is performed at retail outlets serving the final consumer, with proportional allocation of the number of samples to the population of the
geographical region (NUTS-3 area) accounting for at least 80% of the national population, to test for the presence and assess the prevalence of ESBL-/AmpC-/carbapenemase-producing *E. coli*.

It is recommended that these technical specifications be reassessed and updated as needed in the light of the evolution of the trade flows observed in the TRACES statistics, the results of the first monitoring campaigns and the trends observed in the constantly emerging and evolving area of AMR.
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1. Introduction

The Commission adopted in June 2017 a new European One Health action plan against Antimicrobial Resistance (AMR) which provides a framework for continued, more extensive action to reduce the emergence and spread of AMR and to increase the development and availability of new effective antimicrobials inside and outside the EU. Under this new One Health action plan, in order to strengthen the One Health surveillance and reporting of AMR and antimicrobial use, the Commission is committed to review the EU implementing legislation on monitoring AMR in zoonotic and commensal bacteria in farm animals and food, to take into account new scientific developments and data collection needs.

General requirements on the monitoring and reporting of AMR in zoonotic and commensal bacteria has been newly laid down in the Commission Implementing Decision 2020/1729, notably, technical requirements regarding the sampling framework, including indications on the origin of the isolates, the sampling frequency, the sample size and the sample collection. General indications on the laboratory analytical methods for antimicrobial susceptibility testing and data reporting are also provided in the same legislation.

Nevertheless, there is a need for harmonised randomisation procedures for AMR monitoring in samples collected at different stages of the food production chain. The EC has therefore issued a specific mandate to EFSA to provide practical guidance for risk managers, in the different Member States (MSs), for the planning of AMR monitoring programmes based on randomised sampling designs foreseen by Decision 2020/1729. The national competent authority (NCA) is responsible for ensuring the randomisation of the sampling scheme and its correct implementation.

This scientific report provides a rationale and harmonised procedures for randomised sampling for monitoring AMR yielding comparable data. Some proposals made in this report reinforce a number of recommendations already made elsewhere.

1.1. Background and Terms of Reference as provided by the EC

1.1.1. Background

In accordance with Directive 2003/99/EC on monitoring of zoonoses and zoonotic agents Member States must ensure that monitoring provides comparable data on the occurrence of AMR in zoonotic agents and, in so far as they present a threat to public health, other agents. In particular, Member States must ensure that this monitoring provides relevant information at least with regard to a representative number of isolates of Salmonella spp., Campylobacter jejuni and Campylobacter coli from cattle, pigs and poultry and food of animal origin derived from these species.

Commission Implementing Decision 2013/652/EC, implementing Directive 2003/99/EC, lays down detailed and harmonised rules for the monitoring and reporting of AMR, which are applicable from 2014 until the end of 2020. This Decision is based on technical specification documents issued by the EFSA in 2012, providing guidance on the harmonised monitoring of AMR in different bacterial populations of interest.

Furthermore, the EFSA published in 2014 detailed technical specifications on randomised sampling for harmonised monitoring of AMR. These specifications provided a rationale and harmonised practical procedures for randomised sampling of animal and meat samples at different stages of the food chain, yielding representative and comparable data. This led to greater harmonisation and better comparability of data on AMR.

The Commission is now preparing and discussing with Member States a new Commission Implementing Decision on harmonised monitoring of AMR in zoonotic and commensal bacteria from food-producing animals and food. This draft Decision intends to repeal and replace, as from 1 January 2021, the provisions of the current Commission Implementing Decision 2013/652/EU and is based on the recommendations of the EFSA’s scientific report of 5 June 2019 on technical specifications on harmonised monitoring of AMR in zoonotic and indicator bacteria from food-producing animals and food.

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1 COM (2017) 0339.
2 OJ L325, 12.12.2003, p. 31-40.
3 OJ L303, 14.11.2013, p. 26.
4 EFSA Journal 2012; 10(6):2742 and EFSA Journal 2012; 10(10):2897.
5 EFSA Journal 2014;12(5):3686.
More specifically, the draft Decision brings some adaptations to the current AMR monitoring and reporting system as laid down in Decision 2013/652/EU to respond more effectively to the constantly evolving threat of AMR and ensure continuity in assessing future trends in AMR after 2020. These adaptations concern mainly the food-producing animal populations or food categories to be sampled, the sampling design to be followed, the bacterial species to be tested for AMR and the analytical methods to be used by laboratories in charge of testing for AMR. It also aims to minimise as much as possible the burden for the competent authorities in Member States, notably by addressing known implementation issues and focusing AMR monitoring on biological samples or bacterial isolates collected in the framework of already existing national control programmes. As AMR is a global threat that can easily spread across borders, the draft Decision provides for that certain fresh meats imported into the Union are also subjected to AMR monitoring requirements in order to improve coordination and gain knowledge to help reducing AMR impact globally.

For that purpose, it would be useful to have scientific and technical assistance from EFSA on updated harmonised randomisation procedures for AMR monitoring in food-producing animals and food as from 2021.

1.1.2. Terms of Reference as provided by the EC

In accordance with Article 31 of Regulation (EC) No 178/2002, and in light of the experience acquired during the 2014–2019 AMR monitoring campaigns and taking into account the latest version of the draft Commission Implementing Decision on harmonised monitoring of AMR intended to be applicable as from 2021, EFSA is requested to provide scientific and technical assistance by proposing:

1) Updated randomisation procedures for AMR monitoring, where necessary, at the following stages of the food production chain:

   • in flocks of laying hens, broilers and fattening turkeys for samples collected in the framework of the national control programmes provided for in Article 5 of Regulation (EC) No 2160/2003;

   • in slaughterhouses for samples of caecal content taken from broilers, fattening turkeys, fattening pigs and bovine animals under 1 year of age;

   • at retail for samples of fresh meat from broilers, turkeys, pigs and bovine animals.

2) Randomisation procedures for AMR monitoring at border control posts for samples of fresh meat from broilers, turkeys, pigs and bovine animals.

2. Rationale for sampling design for monitoring AMR

2.1. Representative randomised sampling

Isolates which are tested for antimicrobial susceptibility should derive from active monitoring programmes so that the determination of bacterial prevalence in the studied animal populations, whether *Salmonella*, *Campylobacter* or indicator bacteria, as well as the prevalence of resistant bacteria can be ensured. Bacterial isolates tested for antimicrobial susceptibility should originate from healthy animals sampled from randomly selected epidemiological units, whether poultry flocks or slaughter batches randomly selected within the slaughterhouses. Randomised sampling strategies should be emphasised, allowing for proper statistical data analysis and reducing the effect of sampling bias. A random sample in each animal population targeted ensures the representativeness of the entire population and reflects variability in managerial and hygienic practices in holdings and different country regions. An approximately equal distribution of the collected samples over the year enables the different seasons to be covered.

2.2. Sampling frequency and targeted routine monitoring

For the sake of continuity, the sampling is performed consistently on a biennial basis in the combinations of interest. The biennial monitoring has been acknowledged as a good compromise between scientific needs and MS capacities. Annual testing for the monitoring of AMR may still allow earlier detection of evolution in temporal trends, and in those MSs where the epidemiological situation

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6 OJ L 325, 12.12.2003, p. 1–15.
7 If AMR in bacteria from diseased animals is also monitored, these susceptibility results should be reported separately.
requests a more sensitive detection of emerging AMR and trends in the combinations of interest, a switch towards a yearly monitoring may be of relevance.

2.3. Sampling time

An even distribution of the collected samples over the year enables all seasons to be covered. For practical reasons, and to allow for more flexibility in implementing sampling plans, it is proposed to construct the generic proportional allocation in quarters rather than months, although this approach does not hamper performing the randomised sampling on a monthly basis.

2.4. Selection strategy

Samples/isolates are collected according to two different selection strategies, ‘prospective sampling’ and ‘retrospective sampling’.

2.4.1. Prospective sampling

Prospective sampling allows for active monitoring of AMR in bacteria isolated from (1) caecal samples of broilers, fattening turkeys, fattening pigs and bovines under 1 year of age at the slaughterhouse, and (2) samples of fresh broiler meat, turkey meat, pig meat and bovine meat at retail. The representative sampling of caecal samples performed at the slaughterhouse is emphasised, as it increases the chance of obtaining bacterial isolates and is a cost-effective way to collect samples in most of the MS. In the case of prospective sampling, it is expected that MS plan in advance a sampling plan covering the whole year (or most of it) in accordance with the guidelines provided in this report, so that a sufficient number of representative samples is collected evenly throughout the year to eventually obtain the numbers of isolates required by the legislation (e.g. samples to be collected equally split by quarters of the year).

2.4.2. Retrospective sampling

Retrospective sampling allows for active monitoring of AMR in Salmonella spp. isolates obtained from census sampling of laying hen, broiler and fattening turkey flocks, sampled within the framework of the national control programmes (NCP) (as foreseen by Regulation (EC) No 2160/2003).

In the case of retrospective sampling, advantage is taken of Salmonella spp. isolate collections already available from the competent authority (CA). In this case, MS are, therefore, not expected to prepare an additional sampling campaign, but rather to apply the guidelines provided in this report to retrospectively select representative isolates among the ones already available. This should be done at regular intervals during the year (e.g. quarters), so to ensure an even distribution throughout the year of the isolates tested for susceptibility to antimicrobials.

3. Generic stratified sampling approach and sampling plans

The simple and robust randomised sampling procedure currently in place, mostly relying on a stratified sampling approach with proportional allocation of the sample numbers per strata, is reinforced. The general characteristics of the proportional stratified sampling approach, applied to the prospective sampling plans of samples and the retrospective sampling plans of isolates, as foreseen by the Commission Implementing Decision 2020/1729, are briefly presented in Table 1. It illustrates stratified sampling concepts, such as strata, proportional allocation, epidemiological unit, to the sampling plans requested by the legislation. Detailed functional indications about every specific sampling plan are provided in subsequent sections of this report.

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8 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.

9 Stratified sampling is a method of sampling from a population which involves, in the first step, the division of a population into smaller groups known as strata. The strata are formed based on members’ shared attributes or characteristics. In the second step, a random sample from each stratum is taken in a number proportional to the stratum’s size when compared to the population. These subsets of the strata are then pooled to form a random sample. In practice, stratified random sampling is typically employed in large-scale surveys/routine monitoring to reduce some of the logistical costs associated with collecting information from a sample.
3.1. Generic proportionate stratified sampling approach

3.1.1. Generic stratified sampling process

The process of stratified sampling for a particular sampling plan in a given MS is depicted in Figure 1.
### 3.1.2. Identification and Selection of Strata

Once the targeted population and the elements of the sampling procedure, such as strata and epidemiological units (EpiU), have been identified, the number of strata from which to sample (K) is subsequently selected according to the ‘capacity’ of the stratum. Depending on the sampling plan considered, the ‘capacity’ of the stratum may be (1) the annual throughput of the slaughterhouse in the previous year, (2) the number of inhabitants in the NUTS-3 region, (3) number of relevant isolates obtained in the previous year and stored in the isolate collection of the laboratory in an MS.

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**Figure 1:** Flow chart presenting the generic process of stratified sampling

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(a): The strata for which cumulative proportion is larger than 0.6 but smaller than 0.7 are selected, respectively.

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### 3.1.2. Identification and Selection of Strata

Once the targeted population and the elements of the sampling procedure, such as strata and epidemiological units (EpiU), have been identified, the number of strata from which to sample (K) is subsequently selected according to the ‘capacity’ of the stratum. Depending on the sampling plan considered, the ‘capacity’ of the stratum may be (1) the annual throughput of the slaughterhouse in the previous year, (2) the number of inhabitants in the NUTS-3 region, (3) number of relevant isolates obtained in the previous year and stored in the isolate collection of the laboratory in an MS.
representing 60%/80% of the total throughput, inhabitants or isolates collected in the MS once sorted by their capacity for the case of slaughterhouse or laboratories or inhabitants in the different regions.

### 3.1.3. Proportional allocation of sample size per strata and per quarter

Next to the selection of the strata from which to sample, the number of isolates/samples to be taken for each stratum will be proportional to the throughput, inhabitants or previous isolates collected in the laboratories, considering the total number of isolates/samples to be collected in a year divided into four quarters, further assuming 5% of potential missingness (missing data), the required number of isolates/samples per stratum should be inflated 5% in order to achieve the appropriate number of isolates/samples and power. In the case where the number of isolates/samples to be sampled is N, then the number of isolates/samples to be sampled by quarter for each stratum will be calculated as N divided by 4, further multiplied by 1.05 and then multiplied by the allocation proportion previously described (X_{N.Epi.K}).

To account for issues that may be encountered in a number of special cases, scenarios have been developed and are illustrated numerically to guide the process of proportional allocation as shown below, always considering simple random sampling within each of the strata. These numerical illustrations of proportional allocation are performed considering that:

- the total number of samples/isolates to be collected in a year is equal to 170, and thus, the number of samples to be collected per quarter (without considering any potential missingness (missing data)) should therefore equal at least 43;
- the sampling plan focuses on those strata ensuring a representation of at least 60%\(^{10}\) of the population (cumulative proportion is larger than 0.6 but smaller than 0.8), as it is foreseen by the legislation for the sampling of caecal samples at the slaughterhouse.

Nevertheless, while calculating the number of caecal samples to be taken at slaughter, it is advisable to account for the prevalence of the zoonotic bacteria and any potential missingness (missing data).

#### 3.1.3.1. Scenario I

Under Scenario I, it is considered that the number of isolates/samples available/collected per quarter in each of the selected strata (Y_{N.Epi.K}) is larger than the allocated number of isolates/samples to be sampled (X_{N.Epi.K}) (Table 2).

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\(^{10}\) In the case where the plan should ensure a representation of at least 80% of the population, the numerical calculation should be adapted accordingly.
3.1.3.2. Scenario II

Under Scenario II, it is considered that the number of isolates/samples available/collected in the quarter in some (at least one) each of the selected strata (YN.Epi.K) is lower than the allocated number of isolates/samples to be sampled (XN.Epi.K), but the total number of available isolates/samples for the selected strata is equal to the required number of isolates/samples to be sampled; thus, all available isolates/samples are taken (Table 3).

**Table 2:** Numerical illustration of proportional allocation under scenario I(a),(b),(c)

| MS   | Strata | Stratum capacity | Proportion | Cumulative proportion | Allocation proportion (YN.Epi.K) | Samples per quarter (XN.Epi.K) | Sample unit available (YN.Epi.K) | Samples taken (c) |
|------|--------|------------------|------------|-----------------------|-------------------------------|---------------------------------|-------------------------------|------------------|
| Country X P | 66,000,000 | 0.1346 | 0.1346 | 0.2075 | 10 | 15 | 10 |
| Country X G | 65,000,000 | 0.1326 | 0.2672 | 0.2044 | 10 | 16 | 10 |
| Country X M | 54,000,000 | 0.1101 | 0.3773 | 0.1698 | 8 | 9 | 8 |
| Country X Q | 46,000,000 | 0.0938 | 0.4711 | 0.1447 | 7 | 17 | 7 |
| Country X O | 46,000,000 | 0.0938 | 0.5649 | 0.1447 | 7 | 12 | 7 |
| Country X D | 41,000,000 | 0.0836 | 0.6485 | 0.1289 | 6 | 9 | 6 |
| Country X K | 39,000,000 | 0.0795 | 0.7280 | -- | -- | -- | -- |
| Country X C | 27,000,000 | 0.0551 | 0.7831 | -- | -- | -- | -- |
| Country X E | 24,000,000 | 0.0489 | 0.8320 | -- | -- | -- | -- |
| Country X A | 22,000,000 | 0.0449 | 0.8769 | -- | -- | -- | -- |
| Country X F | 19,000,000 | 0.0387 | 0.9156 | -- | -- | -- | -- |
| Country X L | 16,000,000 | 0.0326 | 0.9482 | -- | -- | -- | -- |
| Country X J | 13,000,000 | 0.0265 | 0.9747 | -- | -- | -- | -- |
| Country X H | 12,000,000 | 0.0245 | 0.9992 | -- | -- | -- | -- |
| Country X B | 259,000 | 0.0005 | 0.9997 | -- | -- | -- | -- |
| Country X R | 30,000 | 0.0001 | 0.9998 | -- | -- | -- | -- |
| Country X N | 28,000 | 0.0001 | 0.9999 | -- | -- | -- | -- |
| Country X I | 25,000 | 0.0001 | 1 | -- | -- | -- | -- |
| Total | 18 | 490,342,000 | 1 | 1 | 48 | 78 | 48 |

(a): The calculation is based on the conventions that the total number of samples/isolates to be sampled in a year is equal to 170 and the number of samples/isolates to be sampled per quarter is equal to 43, without accounting for any bacteria prevalence nor considering potential missingness (missing data).

(b): Colour legend:
- Green Cells: Selected stratum representing 60% of the total throughput, inhabitants or isolates collected in the Member State.
- Orange Cells: Stratum for which the available samples/isolates is larger than the number of samples/isolates that should be sampled.

(c): Final adjustments accounting for the prevalence of the bacteria and rate of recovery after storage are not addressed in this illustration.
3.1.3.3. Scenario III

Under Scenario III, the number of isolates/samples available/collected in the quarter in some (at least one) of the selected strata (YN.Epi.K) is not larger than the allocated number of isolates/samples to be sampled (XN.Epi.K), but the total number of available isolates/samples is larger than the required number of isolates/samples to be sampled (Table 4). Next, from those strata (K') where (XN.Epi.K' ≥ YN.Epi.K) all YN.Epi.K' will be taken, and the rest of the samples to be collected (sr = \( \sum_{j \in K} X_{N.Epi,j} - \sum_{h \in K'} Y_{N.Epi,h} \)) will be redistributed among the remainder of the strata (K''). The distribution among the remainder strata will be the minimum value between the samples available (YN.Epi.K'') and the srmultiplied by the allocation proportion for each EpiU not yet sampled (K'') divided by the sum of all allocation proportions of the units not yet sampled, the mathematical expression would be:

\[
\min \left( Y_{N.Epi,h} \cdot sr \cdot \frac{\sum_{h \in K''} Y_{N.Epi,h}}{\sum_{h \in K''}} \right), \quad \forall h \in K''.
\]

### Table 3: Numerical illustration of proportional allocation under Scenario II(a),(b),(c)

| MS    | Strata | Stratum capacity | Proportion | Cumulative proportion | Allocation proportion (\( \pi_{N.Epi.K} \)) | Samples per quarter (\( X_{N.Epi.K} \)) | Sample unit available (\( Y_{N.Epi.K} \)) | Samples taken(c) |
|-------|--------|------------------|------------|-----------------------|-----------------------------------------------|------------------------------------------|------------------------------------------|------------------|
| Country X P | 66,000,000 | 0.1346 | 0.2694 | 0.2075 | 10 | 12 | 12 |
| Country X G | 65,000,000 | 0.1326 | 0.3920 | 0.2044 | 10 | 8 | 8 |
| Country X M | 54,000,000 | 0.1101 | 0.5021 | 0.1698 | 8 | 7 | 7 |
| Country X Q | 46,000,000 | 0.0938 | 0.5959 | 0.1447 | 7 | 9 | 9 |
| Country X O | 46,000,000 | 0.0938 | 0.6798 | 0.1447 | 7 | 7 | 7 |
| Country X D | 41,000,000 | 0.0836 | 0.6485 | 0.1289 | 6 | 5 | 5 |
| Country X K | 39,000,000 | 0.0795 | 0.7280 | | | | |
| Country X C | 27,000,000 | 0.0551 | 0.7831 | | | | |
| Country X E | 24,000,000 | 0.0489 | 0.8320 | | | | |
| Country X A | 22,000,000 | 0.0449 | 0.8769 | | | | |
| Country X F | 19,000,000 | 0.0387 | 0.9156 | | | | |
| Country X L | 16,000,000 | 0.0326 | 0.9482 | | | | |
| Country X J | 13,000,000 | 0.0265 | 0.9745 | | | | |
| Country X H | 12,000,000 | 0.0245 | 0.9992 | | | | |
| Country X B | 259,000 | 0.0005 | 0.9997 | | | | |
| Country X R | 30,000 | 0.0001 | 0.9998 | | | | |
| Country X N | 28,000 | 0.0001 | 0.9999 | | | | |
| Country X I | 25,000 | 0.0001 | 1 | | | | |
| Total: | 18 | 490,342,000 | 1 | 1 | 48 | 48 | 48 |

(a): The calculation is based on the conventions that the total number of samples/isolates to be sampled in a year is equal to 170 and the number of samples/isolates to be sampled per quarter is equal to 43, without accounting for any bacteria prevalence nor considering potential missingness (missing data).

(b): Colour legend:
- Green Cells: Selected stratium representing 60% of the total throughput, inhabitants or isolates collected in the Member State.
- Yellow Cells: Stratum for which the available samples/isolates is smaller than the number of samples/isolates that should be sampled.
- Orange Cells: Stratum for which the available samples/isolates is larger than the number of samples/isolates that should be sampled.

(c): Final adjustments accounting for the prevalence of the bacteria and rate of recovery after storage are not addressed in this illustration.

3.1.3.3. Scenario III

Under Scenario III, the number of isolates/samples available/collected in the quarter in some (at least one) of the selected strata (YN.Epi.K) is not larger than the allocated number of isolates/samples to be sampled (XN.Epi.K), but the total number of available isolates/samples is larger than the required number of isolates/samples to be sampled (Table 4). Next, from those strata (K') where (XN.Epi.K' ≥ YN.Epi.K) all YN.Epi.K' will be taken, and the rest of the samples to be collected (sr = \( \sum_{j \in K} X_{N.Epi,j} - \sum_{h \in K'} Y_{N.Epi,h} \)) will be redistributed among the remainder of the strata (K''). The distribution among the remainder strata will be the minimum value between the samples available (YN.Epi.K'') and the srmultiplied by the allocation proportion for each EpiU not yet sampled (K'') divided by the sum of all allocation proportions of the units not yet sampled, the mathematical expression would be:
### 3.1.3.4. Scenario IV

Under Scenario IV, it is considered that the number of isolates/samples available/collected quarterly in some (at least one) each of the selected strata (YN.Epi.K) is lower than the allocated number of isolates/samples to be sampled (XN.Epi.K), and the total number of available isolates/samples is lower than the required number of isolates/samples to be sampled; thus, all available isolates/samples from the selected strata are selected and the number of isolates/samples that need to be still sampled should come from the remainder of the strata (the 40% initially excluded), considering the original ranking of strata performed on the basis of the capacity of stratum (i.e. throughput, inhabitants or isolates collected) (Table 5).

### Table 4: Numerical illustration of proportional allocation under Scenario III(a),(b),(c)

| MS  | Strata | Capacity   | Capacity (%) | Cumulative proportion | Allocation proportion (\(\pi_{N,Epi.K}\)) | Samples per quarter (\(X_{N,Epi.K}\)) | Sample unit available (\(Y_{N,Epi.K}\)) | Samples taken (\(c\)) |
|-----|--------|------------|--------------|-----------------------|--------------------------------------------|----------------------------------------|----------------------------------------|-----------------------|
| Country X | P       | 66,000,000 | 0.1346       | 0.1346                | 0.2075                                     | 10                                     | 15                                     | 15                    |
| Country X | G       | 65,000,000 | 0.1326       | 0.2672                | 0.2044                                     | 10                                     | 6                                      | 6                     |
| Country X | M       | 54,000,000 | 0.1101       | 0.3773                | 0.1698                                     | 8                                      | 7                                      | 7                     |
| Country X | Q       | 46,000,000 | 0.0938       | 0.4711                | 0.1447                                     | 7                                      | 17                                     | 12                    |
| Country X | O       | 46,000,000 | 0.0938       | 0.5649                | 0.1447                                     | 7                                      | 3                                      | 3                     |
| Country X | D       | 41,000,000 | 0.0836       | 0.6485                | 0.1289                                     | 6                                      | 5                                      | 5                     |
| Country X | K       | 39,000,000 | 0.0795       | 0.7280                |                                            |                                        |                                        |                        |
| Country X | C       | 27,000,000 | 0.0551       | 0.7831                |                                            |                                        |                                        |                        |
| Country X | E       | 24,000,000 | 0.0489       | 0.8320                |                                            |                                        |                                        |                        |
| Country X | A       | 22,000,000 | 0.0449       | 0.8769                |                                            |                                        |                                        |                        |
| Country X | F       | 19,000,000 | 0.0387       | 0.9156                |                                            |                                        |                                        |                        |
| Country X | L       | 16,000,000 | 0.0326       | 0.9482                |                                            |                                        |                                        |                        |
| Country X | J       | 13,000,000 | 0.0265       | 0.9747                |                                            |                                        |                                        |                        |
| Country X | H       | 12,000,000 | 0.0245       | 0.9992                |                                            |                                        |                                        |                        |
| Country X | B       | 259,000    | 0.0005       | 0.9997                |                                            |                                        |                                        |                        |
| Country X | R       | 30,000     | 0.0001       | 0.9998                |                                            |                                        |                                        |                        |
| Country X | N       | 28,000     | 0.0001       | 0.9999                |                                            |                                        |                                        |                        |
| Country X | I       | 25,000     | 0.0001       | 1                     |                                            |                                        |                                        |                        |
| Total    |         | 490,342,000| 1            | 1                     | 48                                         | 53                                     | 48                                     |                        |

(a): The calculation is based on the conventions that the total number of samples/isolates to be sampled in a year is equal to 170 and the number of samples/isolates to be sampled per quarter is equal to 43, without accounting for any bacteria prevalence nor considering potential missingness (missing data).

(b): Colour legend:
- Green Cells: Selected stratum representing 60% of the total throughput, inhabitants or isolates collected in the Member State.
- Yellow Cells: Stratum for which the available samples/isolates is smaller than the number of samples/isolates that should be sampled.
- Orange Cells: Stratum for which the available samples/isolates is larger than the number of samples/isolates that should be sampled.

(c): Final adjustments accounting for the prevalence of the bacteria and rate of recovery after storage are not addressed in this illustration.

### 3.1.3.4. Scenario IV

Under Scenario IV, it is considered that the number of isolates/samples available/collected quarterly in some (at least one) each of the selected strata (YN.Epi.K) is lower than the allocated number of isolates/samples to be sampled (XN.Epi.K), and the total number of available isolates/samples is lower than the required number of isolates/samples to be sampled; thus, all available isolates/samples from the selected strata are selected and the number of isolates/samples that need to be still sampled should come from the remainder of the strata (the 40% initially excluded), considering the original ranking of strata performed on the basis of the capacity of stratum (i.e. throughput, inhabitants or isolates collected) (Table 5).
3.1.3.5. Scenario V

Under Scenario V, it is considered that the number of isolates/samples available/collected in the quarter in some of the selected strata \( (Y_{N.Epi,K}) \) is lower than the allocated total number of isolates/samples to be sampled \( (X_{N.Epi,K}) \) and the rest of the strata cannot be sampled (for economical or practical reasons), or the number of samples in the remainder of the strata (the 40% initially excluded) is less than the number of isolates/samples that still need to be sampled, then the next quarter(s) the number of samples to be taken should compensate for the number of samples that were not collected during the previous quarter(s).

Take all isolates/samples from the selected strata \( (N_q = 45) \) and the number of isolates/samples to be taken in the next quarter will be equal to the original amount plus the number of samples/isolates that were not taken in the previous quarter e.g. in Table 6 two possible solutions would be:

1) To compensate for the number of isolates/samples that were not collected the previous quarter directly in the next quarter, for instance the number of isolates/samples to be collected in the next quarter would be \( 48 + (48 - N_q) = 48 + (48 - 45) = 51 \).

2) The number of samples/isolates to be collected in the following quarters (let \( n_{\text{quarter}} \) represent the number of quarter still to be sampled, assuming that \( n_{\text{quarter}} = 3 \) would be adjusted as

\[
\frac{48 \times n_{\text{quarter}} + (48 - N_q)}{n_{\text{quarter}}} = 48 + \frac{(48 - N_q)}{n_{\text{quarter}}} = 48 + \frac{(48 - 45)}{3} = 49.
\]

Table 5: Numerical illustration of proportional allocation under Scenario IV(a),(b),(c)

| MS    | Strata | Stratum capacity | Proportion | Cumulative proportion | Allocation proportion \( (\hat{x}_{N.Epi,K}) \) | Samples per quarter \( (X_{N.Epi,K}) \) | Sample unit available \( (Y_{N.Epi,K}) \) | Samples taken \( (c) \) |
|-------|--------|------------------|------------|-----------------------|-----------------------------------------------|------------------------------------------|------------------------------------------|-------------------------|
| Country X | P      | 66,000,000       | 0.1346     | 0.1346                | 0.2075                                        | 10                                       | 13                                       | 13                      |
| Country X | G      | 65,000,000       | 0.1326     | 0.2672                | 0.2044                                        | 10                                       | 6                                        | 6                       |
| Country X | M      | 54,000,000       | 0.1101     | 0.3773                | 0.1698                                        | 8                                        | 7                                        | 7                       |
| Country X | Q      | 46,000,000       | 0.0938     | 0.4711                | 0.1447                                        | 7                                        | 11                                       | 11                      |
| Country X | O      | 46,000,000       | 0.0938     | 0.5649                | 0.1447                                        | 7                                        | 3                                        | 3                       |
| Country X | D      | 41,000,000       | 0.0836     | 0.6485                | 0.1289                                        | 6                                        | 5                                        | 5                       |
| Country X | K      | 39,000,000       | 0.0795     | 0.7280                |                                               | 2                                        | 2                                        |                         |
| Country X | C      | 27,000,000       | 0.0551     | 0.7831                |                                               | 6                                        | 1                                        |                         |
| Country X | E      | 24,000,000       | 0.0489     | 0.8320                |                                               | 3                                        | 0                                        |                         |
| Country X | A      | 22,000,000       | 0.0449     | 0.8769                |                                               | 4                                        | 0                                        |                         |
| Country X | F      | 19,000,000       | 0.0387     | 0.9156                |                                               | 2                                        | 0                                        |                         |
| Country X | L      | 16,000,000       | 0.0326     | 0.9482                |                                               | 9                                        | 0                                        |                         |
| Country X | J      | 13,000,000       | 0.0265     | 0.9747                |                                               | 5                                        | 0                                        |                         |
| Country X | H      | 12,000,000       | 0.0245     | 0.9992                |                                               | 2                                        | 0                                        |                         |
| Country X | B      | 259,000          | 0.0005     | 0.9997                |                                               | 3                                        | 0                                        |                         |
| Country X | R      | 30,000           | 0.0001     | 0.9998                |                                               | 4                                        | 0                                        |                         |
| Country X | N      | 28,000           | 0.0001     | 0.9999                |                                               | 8                                        | 0                                        |                         |
| Country X | I      | 25,000           | 0.0001     | 1                     |                                               | 7                                        | 0                                        |                         |
| Total   |        | 490,342,000      | 1          | 1                     | 48                                           | 100                                      | 48                                       |

(a): The calculation is based on the conventions that the total number of samples/isolates to be sampled in a year is equal to 170 and the number of samples/isolates to be sampled per quarter is equal to 43, without accounting for any bacteria prevalence or considering potential missingness (missing data).

(b): Colour legend:
Green Cells: Selected stratum representing 60% of the total throughput, inhabitants or isolates collected in the MS.
Yellow Cells: Stratum for which the available samples/isolates is smaller than the number of samples/isolates that should be sampled.
Orange Cells: Stratum for which the available samples/isolates is larger than the number of samples/isolates that should be sampled.

(c): Final adjustments accounting for the prevalence of the bacteria and rate of recovery after storage are not addressed in this illustration.

3.1.3.5. Scenario V

Under Scenario V, it is considered that the number of isolates/samples available/collected in the quarter in some of the selected strata \( (Y_{N.Epi,K}) \) is lower than the allocated total number of isolates/samples to be sampled \( (X_{N.Epi,K}) \) and the rest of the strata cannot be sampled (for economical or practical reasons), or the number of samples in the remainder of the strata (the 40% initially excluded) is less than the number of isolates/samples that still need to be sampled, then the next quarter(s) the number of samples to be taken should compensate for the number of samples that were not collected during the previous quarter(s).

Take all isolates/samples from the selected strata \( (N_q = 45) \) and the number of isolates/samples to be taken in the next quarter will be equal to the original amount plus the number of samples/isolates that were not taken in the previous quarter e.g. in Table 6 two possible solutions would be:

1) To compensate for the number of isolates/samples that were not collected the previous quarter directly in the next quarter, for instance the number of isolates/samples to be collected in the next quarter would be \( 48 + (48 - N_q) = 48 + (48 - 45) = 51 \).

2) The number of samples/isolates to be collected in the following quarters (let \( n_{\text{quarter}} \) represent the number of quarter still to be sampled, assuming that \( n_{\text{quarter}} = 3 \) would be adjusted as

\[
\frac{48 \times n_{\text{quarter}} + (48 - N_q)}{n_{\text{quarter}}} = 48 + \frac{(48 - N_q)}{n_{\text{quarter}}} = 48 + \frac{(48 - 45)}{3} = 49.
\]
3.1.4. Accounting for the prevalence and recovery rate of the bacteria

The number of samples to be collected from each animal population in order to achieve the number of isolates required depends on the prevalence of the bacteria considered (C. jejuni, C. coli, E. coli, E. faecium and E. faecalis). The CA should estimate the number of samples to be collected in order to obtain the required number of bacterial isolates, given the prevalence of those particular bacteria in caecal samples of the specific animal populations: the predominant Campylobacter species in broilers, fattening turkeys and calves and C. coli in fattening pigs. Assuming that the sample is selected randomly and that the prevalence in the animals slaughtered in the selected slaughterhouses is similar to the national prevalence, the number of samples to be collected can be calculated as the inverse of the prevalence. For example, if the prevalence was 70%, the number of samples to be collected in order to ensure the collection of 170 isolates would be equal to 242 (170/0.7 = 242).

Regarding Campylobacter, in the particular case of those MSs where a particularly marked seasonality in the prevalence occurs, it may be desirable, for cost-effectiveness reasons, to focus on the quarters (seasons) where the prevalence is not low to very low and the chance of isolating Campylobacter higher. The intention is to primarily ensure a sufficient number of isolates to assess antimicrobial resistance levels.

For indicator E. coli, which is highly prevalent, the number of samples to be collected would be slightly higher than the number of isolates required, as it can be assumed that these bacteria are almost constantly recovered from animal caecal samples (prevalence rates/isolation rates ranging between 95% and 100%).

For indicator Enterococcus spp., the monitoring of AMR may be performed on a voluntary basis. The expected prevalence of enterococci is typically much lower, e.g. around 50% in caecal samples from broilers, and this should be accounted for. The number of samples could be reduced, if one
isolate of each of the species \textit{E. faecium} and \textit{E. faecalis}, from the same sample, is taken. For this purpose, the samples collected could be tested for the presence of \textit{Enterococcus} spp. and more than one isolated strain of \textit{Enterococcus} spp. per sample (typically between 1 and 5) stored to be subsequently speciated by polymerase chain reaction (PCR). Among the \textit{E. faecalis} and \textit{E. faecium} isolates available, only one per species and per sample should be finally tested for susceptibility and the results for \textit{E. faecium} and \textit{E. faecalis} reported separately. Low numbers of \textit{E. faecium} and \textit{E. faecalis} isolates per animal population would mean that for these animal populations, there is a decreased precision of the estimate of the proportion of resistance and it would be more difficult to detect trends over time.

As previously indicated, in order to also take into account a 5% missingness (missing data), the number of yearly samples to be collected should be further inflated by 5%, e.g. 179 samples \((170 \times 1.05 = 178.5)\) or 254 samples \((242 \times 1.05 = 254)\) in the examples given above. As the strain recovery rate after storage may be less than 100%, the number of yearly samples to be collected should be further inflated to offset this, based on the experience of the laboratories, for e.g. by an additional 2%. The CA may take into account varying local situations at national level and introduce inflation factors in calculation for bacterial species where necessary and also possibly adapt the values of the inflation factors according to the field situation.

3.1.5. Simple random sampling of samples/isolates within the stratum

Simple random sampling (SRS) is the simplest form of drawing elements from a targeted population. It involves drawing elements successively such that each population member has equal and a non-zero probability of being selected. An SRS procedure is applied in order to select an adequate number of isolates/samples to be taken by quarter. If, in practice, it is more convenient for the central and local CAs, the SRS may also be planned and performed by month. For certain sampling plans, it may be necessary to perform a series of SRS processes, for example, regarding the sampling of caecal samples at slaughter, working days should be selected first, then batch(es)/lot(s) of carcasses originating from the same epidemiological unit (flock or herd) on the selected days, and finally one carcase (or a number of carcases in the case of a grouped sample) from the selected batch(es).

Considering a list with \(N\) elements (e.g. isolates in isolate collection or days/working days in a month), a sample of \(n\) elements should be drawn. Each list element is assigned a number, say, \(1, \ldots, N\), and the selection can proceed the following way: (i) randomly select a number between 1 and \(N\) (this can be done with Excel using the = RANDBETWEEN(1, \(N\)) function and many other statistical software). The element corresponding to the selected number is included in the sample and it cannot be selected again, this is referred to as sampling without replacement.

4. Sampling plans of \textit{Salmonella} isolates from primary production of poultry

4.1. Objective

AMR monitoring in \textit{Salmonella} spp. isolates from poultry populations of various production (i.e. laying hens, broilers, fattening turkeys) targeted by national control (and monitoring) programmes (NCPs) of Salmonella is based on Salmonella isolates collected in the framework of these control programmes. For the NCPs of Salmonella in such poultry populations, minimum requirements on the collection of type of material and where the sampling is to take place are already fixed by the EU legislation. An unbiased estimate of the proportion of resistance may be obtained through a sampling frame covering all epidemiological units (flocks) of the national production. This is most readily achieved if Salmonella isolates originate from the NCPs. The epidemiological unit for the various poultry populations concerned (broilers, laying hens and fattening turkeys) is the flock, because most holdings practise all-in-all-out production. It is assumed that \textit{Salmonella} isolates of the same serovar from the same epidemiological unit (poultry flock) show a similar pattern of resistance. To ensure representativeness, susceptibility testing should be done for no more than one isolate per \textit{Salmonella} serovar from the same epidemiological unit/year.

The number of \textit{Salmonella} isolates deriving from the NCPs to be tested per poultry population is 170 (this may be reduced to 85 organisms from poultry and pigs, if production is less than 100,000 tonnes per annum). 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. In the case of
higher number of *Salmonella* isolates available, a random selection of 170 isolates should be performed from the collection of yearly available isolates in the MS. In the case of low prevalence, all the *Salmonella* isolates available - clinical isolates excluded - should be tested for susceptibility.

The objective is to collect and test for antimicrobial susceptibility 170 representative *Salmonella* spp. isolates obtained, respectively, from the populations of laying hen flocks, broiler flocks and fattening turkey flocks in the MS on a yearly basis (whether the sampling was performed by the CA or under its supervision, by the FBO), where possible. In any case, operators should carefully check that the *Salmonella* strains randomly selected and submitted for susceptibility testing originate from different (positive) flocks and, optimally, from different farms.

### 4.2. The delineation of animal populations

Flocks of broilers, laying hens and fattening turkeys (in the MS where more than 10,000 tonnes are slaughtered on a yearly basis) in production between 1 January and 31 December and covered by the *Salmonella* National Control Programme (NCP) are eligible for AMR monitoring in *Salmonella* spp. isolates.

### 4.3. Samples and the sampling designs

Two approaches are advisable, according to the *Salmonella* prevalence in the populations of laying hen flocks, broiler flocks, fattening turkey flocks and the availability of a central database recording the positive flocks in real time.

#### 4.3.1. Approach 1: proportionate stratified sampling of isolates

This approach follows a stratified sampling strategy with proportional allocation within a sampling frame of *Salmonella* spp. strains deriving from the isolate collections available from the official laboratories and/or other laboratories designated by the CA to carry out testing under the NCP requirements\(^\text{11}\) involved in the *Salmonella* NCP. For this purpose, the following parameters are considered when adapting the generic stratified sampling approach to this specific sampling:

- **Strata**: the laboratories involved in the *Salmonella* NCP.\(^\text{12}\)
- **Stratum capacity**: the size of the collection of *Salmonella* strains originating from the animal population examined and isolated within the framework of the NCP in the previous year, available in the laboratory.
- **Epidemiological unit (EpiU)**: For the purpose of this sampling, epidemiological units are represented by flocks of broilers/laying hens/fattening turkeys.

The *Salmonella* spp. strains, respectively, isolated from broiler flocks, laying hen flocks and fattening turkey flocks, covered by the *Salmonella* NCP of the MS and in production from 1 January to 31 December are eligible. It is of note that *S. Gallinarum Pullorum* strains as well as the other *Salmonella* strains isolated within the context of clinical investigations are excluded for the purpose of the AMR monitoring. Each MS shall list all official laboratories and/or other laboratories designated by the CA involved in the testing of samples within the framework of the NCP and rank them by decreasing order by number of eligible *Salmonella* spp. strains isolated from laying hen/broiler/fattening turkey flocks between 1 January to 31 December in the previous year. A sampling plan is designed by the CA at the beginning of the year in order to plan the activity and adapted as necessary in the course of the year so that the required number of representative bacterial isolates can be collected and eventually submitted for susceptibility testing.

The list of participating laboratories accounting for (at least 80% of) the total number of *Salmonella* isolates is then compiled starting with the laboratories with largest isolate collection (to be considered separately per animal population of origin). The isolate sample size for each laboratory in the sampling period is allocated proportionally to the laboratory isolate collection size of the previous year. Every quarter, simple random sampling is performed within the sampling frame of strains (see approach

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\(^{11}\) Designated under Regulation (EC) 2160/2003 article 12(1)(a).

\(^{12}\) As a logistic compromise, it may be acceptable that the sampling plan focus on those laboratories accounting for 80% of the total number of *Salmonella* isolates in the poultry production in question within the MS in the previous year. If the *Salmonella* prevalence in the poultry productions is important and the laboratories involved in the NCPs numerous, it may be envisaged to account for organisational constraints to further limit the strata (laboratories) involved in the sampling plans, provided that a good geographical representation is assured and any particular subpopulations are not systematically included/excluded.
presented in Section 3.1.3), unique per positive flock, up to the selection of the quarter of yearly isolate sample size. Either the sampling frame of *Salmonella* strains in the collections of the laboratories may include only strains unique per positive flock or a check for duplicates per positive flock is carried out after the random selection.

In the particular case where the number of available isolates is lower than the quarter of the yearly isolate sample size, the scenario III, IV and V should apply in order to complement the isolate sample size up to (at least) 170 isolates.

In the case where less than 170 *Salmonella* isolates have been isolated in each of these poultry populations in a given year, all available isolates should be included in AMR monitoring, provided that there is no more than one isolate per *Salmonella* serovar from the same flock (epidemiological unit) per year.

### 4.3.2. Approach 2: simple random sampling of positive flocks

An alternative approach is to perform a simple random sampling (SRS) within the sampling frame of flocks involved in the NCP and which have tested positive for *Salmonella*. In the MS where a national database records the flocks of laying hens/broilers/fattening turkeys tested positive for *Salmonella* spp. (all serovars) in real time, it is advisable to devise the sampling design as a quarterly SRS of the flocks tested positive for *Salmonella*. Every quarter, the number of positive flocks to be randomly selected among the flocks tested positive over the quarter is equal to 43 flocks. The SRS is performed according to the procedure developed in Section 3.1.3.

A unique strain of *Salmonella* spp. (in the case all the isolates recovered belong to the same serovar) or a unique strain per *Salmonella* serovar (in the case the isolate recovered belong to different serovars) recovered from each positive flock randomly selected is submitted for susceptibility testing. If more than one strain has been isolated from the flock, a random selection of a strain among those isolated is performed from the competent laboratory collection and included in the AMR monitoring programme.

If less than 43 flocks tested positive in a given quarter, the total number of strains available is included in the AMR monitoring programme. In this case, it is advisable that the next quarter(s) the number of isolates to be taken should compensate for the number of samples that were not collected during the previous quarter(s), if possible.

### 5. Sampling plans of representative caecal isolates at slaughter

#### 5.1. Objective

AMR monitoring is based on the representative/random collection of caecal samples at the slaughterhouse. Sampling performed at the slaughterhouse is emphasised, as in many of the MSSs, it will be most cost-effective way to collect the samples. At least 60% of the domestic animal population in an MS are included in the sampling frame, meaning that slaughterhouses processing at least 60% of the domestic animals of the relevant animal category (starting with the slaughterhouses of largest throughput) are eligible for sampling.13 An active monitoring programme should be based on random sampling of carcases of healthy animals. The 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. An approximately equal distribution of the collected samples over the year enables the different seasons to be covered.

The epidemiological unit for fattening pigs and bovine animals under 1 year of age is the slaughter batch. Only one representative sample of caecal content per epidemiological unit (e.g. flock, batch of animals sent to the slaughterhouse), derived from 1 carcase, is gathered to account for clustering. Regarding broilers, the representative sample of caecal content collected from a given epidemiological unit should derive from 10 animals, in order to collect enough material to test for the presence of the monitored bacteria.

The objective is to collect and test for antimicrobial susceptibility representative caecal isolates of:

- *E. coli*,
- *C. jejuni* and *C. coli*.

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13 In the case where the tonnage of the domestically produced animals slaughtered represents only a limited fraction of the total tonnage of slaughtered animals, which might make difficult the implementation of the sampling plan, it is advised to select the slaughterhouses involved in the sampling plan by considering the tonnages of domestically produced animals slaughtered, whenever corresponding data are available.
• Extended Spectrum Beta-Lactamase (ESBL)/AmpC/carbapenemase-producing \( E. \) coli, from broilers, fattening turkeys, fattening pigs and bovine animals under 1 year of age,

• \( \text{Salmonella} \) spp., from fattening pigs, except for MSs implementing a national programme for the control of \( \text{Salmonella} \) (approved at the EU level), and from bovine animals under one year of age in those MSs where the national production of meat of those bovine animals is more than 10,000 tonnes per year, and, on a voluntary basis:

• \( E. \text{faecium} \) and \( E. \text{faecalis} \), from broilers, fattening turkeys, fattening pigs and bovine animals under 1 year of age.

The minimum target number of organisms of \( E. \) coli and of the most prevalent species of \( \text{Campylobacter} \) (among \( C. \) coli and \( C. \) jejuni) which should be examined is 170 from each type of domestic animal production considered (this may be reduced to 85 organisms from broilers and pigs, if production corresponding meat is less than 100,000 tonnes per year). Regarding the less prevalent \( \text{Campylobacter} \) species, the organisms isolated while applying the harmonised protocol for isolation recommended by the EURL-\( \text{Campylobacter} \) should be considered up to a maximum of 170 isolates.

Within the context of the implementation of the EU and MSs’ action plans against the threat of AMR, a further decrease in use of antimicrobials in food-producing animals is expected to occur in conjunction with implementing complementary mitigation measures in the coming years, resulting in a decrease in the selective pressure on the emergence and/or occurrence of AMR. In order to ensure sufficient statistical power so that even slight decreases in AMR can be detected, it is therefore recommended that sample size, notably for indicator \( E. \) coli, is reviewed by each MS taking into account their own situation and objectives of reduction of AMR in the light of the simulations presented elsewhere (EFSA, 2019), which also account for the assessment of the occurrence of resistance with sufficient accuracy. It is acknowledged that this approach may lead to an increase in the number of samples to be collected, and that this may require additional resources from the MSs.

The MSs assess which specific combinations of animal populations and bacteria should be sampled according to Decision 2020/1729, what number of isolates/samples should be collected and tested, and in which years. Table 7 can be used as a means to identify the isolates/samples to be collected.

5.2. The delineation of the animal populations

The eligible populations of broilers/fattening turkeys/fattening pigs/bovine animals under 1 year of age cover those domestically produced and slaughtered in the slaughterhouses covering at least 60% of the all broilers/fattening turkeys/fattening pigs/bovine animals under 1 year of age slaughtered in the MS.

It is proposed that domestically produced broilers, fattening turkeys, fattening pigs and bovine animals under 1 year of age may be, respectively, defined:

• Regarding broilers and fattening turkeys, as birds hatched and raised in the MS as well as those imported/traded as day-old chicks into the MS (i.e. hatched abroad).

• Regarding fattening pigs and bovine animals of less than 1 year of age, as those animals produced and slaughtered in the MS and excluding those imported and intended for direct slaughter after importation. Therefore, pigs imported/traded into the MS as post-weaners, growers and fatteners are considered as domestically produced animals. As a general guideline, domestically produced animals should spend at least about 50% of their lifespan in the MS.
Table 7: Animal populations to be sampled for caecal samples at slaughter and number of samples/isolates foreseen per bacteria and per year

| Animal population | Bacteria                                                                 | 2021    | 2022    | 2023    | 2024    | 2025    | 2026    | 2027    |
|-------------------|--------------------------------------------------------------------------|---------|---------|---------|---------|---------|---------|---------|
| Broilers          | *C. jejuni* and *C. coli*(f)                                            | –       | 170 isolates(c) | –       | 170 isolates(c) | –       | 170 isolates(c) | –       |
|                   | *E. coli*                                                               | –       | 170 isolates(c) | –       | 170 isolates(c) | –       | 170 isolates(c) | –       |
|                   | *E. faecalis*, *E. faecium*(b)                                          | –       | 170 isolates(c) | –       | 170 isolates(c) | –       | 170 isolates(c) | –       |
|                   | Enzyme-producing *E. coli*(e)                                           | –       | 300 samples(d) | –       | 300 samples(d) | –       | 300 samples(d) | –       |
| Fattening turkeys\((a)\) | *C. jejuni and C. coli*(f)                                            | –       | 170 isolates | –       | 170 isolates | –       | 170 isolates | –       |
|                   | *E. coli*                                                               | –       | 170 isolates(c) | –       | 170 isolates(c) | –       | 170 isolates(c) | –       |
|                   | *E. faecalis*, *E. faecium*(b)                                          | –       | 170 isolates(c) | –       | 170 isolates(c) | –       | 170 isolates(c) | –       |
|                   | Enzyme-producing *E. coli*(e)                                           | –       | 300 samples(d) | –       | 300 samples(d) | –       | 300 samples(d) | –       |
| Fattening pigs    | *C. coli*                                                               | 170 isolates(c),(g) | –       | 170 isolates(c),(g) | –       | 170 isolates(c),(g) | –       | 170 isolates(c),(g) |
|                   | *Salmonella*                                                            | 170 isolates(c),(g) | –       | 170 isolates(c),(g) | –       | 170 isolates(c),(g) | –       | 170 isolates(c),(g) |
|                   | *E. coli*                                                               | 170 isolates(c) | –       | 170 isolates(c) | –       | 170 isolates(c) | –       | 170 isolates(c) |
|                   | *E. faecalis*, *E. faecium*(b)                                          | 170 isolates(c) | –       | 170 isolates(c) | –       | 170 isolates(c) | –       | 170 isolates(c) |
|                   | Enzyme-producing *E. coli*(e)                                           | 300 samples(d) | –       | 300 samples(d) | –       | 300 samples(d) | –       | 300 samples(d) |
| Bovines < 1 y. of age\((a)\) | *C. jejuni and C. coli*(f)                                            | 170 isolates(g) | –       | 170 isolates(g) | –       | 170 isolates(g) | –       | 170 isolates(g) |
|                   | *Salmonella*                                                            | 170 isolates(g) | –       | 170 isolates(g) | –       | 170 isolates(g) | –       | 170 isolates(g) |
|                   | *E. coli*                                                               | 170 isolates | –       | 170 isolates | –       | 170 isolates | –       | 170 isolates |
|                   | *E. faecalis*, *E. faecium*(b)                                          | 170 isolates(c) | –       | 170 isolates(c) | –       | 170 isolates(c) | –       | 170 isolates(c) |
|                   | Enzyme-producing *E. coli*(e)                                           | 300 samples(d) | –       | 300 samples(d) | –       | 300 samples(d) | –       | 300 samples(d) |

(a): to be sampled only if annual national production (turkey meat, meat of bovine animals under 1 year of age) is greater than 10,000 tonnes per year.

(b): to be sampled on a voluntary basis.

(c): 85 isolates if national annual production is less than 100,000 tonnes per year.

(d): 150 samples if annual national production (broiler meat, turkey meat, pig meat) is less than 100,000 tonnes per year or if production (bovine meat) is less than 50,000 tonnes per year.

(e): Enzyme-producing *E. coli* states for those isolates deriving from specific monitoring of ESBL-/AmpC-/carbapenemase-producing *E. coli* and specific monitoring of CP-producing *E. coli*.

(f): For each MS, the target of 170 isolates applies to the most prevalent species of *Campylobacter*; all isolates of the other *Campylobacter* species (up to a maximum of 170 isolates) that are identified, considering the specification of one isolate per species and epidemiological unit, are to be included.

(g): 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.
5.3. Samples and sampling design

A sampling plan is to be designed by the CA at the beginning of the year (at the latest) in order to ensure that the required number of representative bacterial isolates is collected and eventually submitted for susceptibility testing. For this purpose, the following parameters are considered when adapting the generic stratified sampling approach to this specific sampling:

- **Strata (1st stage):** the slaughterhouses where eligible animals are slaughtered within the MS.
- **Stratum capacity:** the annual throughput of **domestically produced** meat of the slaughterhouse during the previous year or according to the latest data available.\(^{14}\)
- **Strata (2nd stage):** For the purpose of this sampling at slaughter, batches/lots of carcases originating from the same epidemiological unit (flock of broilers/fattening turkeys and slaughter batch of fattening pigs/bovine animals under 1 year of age) are used for a second-stage selection. As indicated below, no more than one caecal content sample (single or pooled)\(^{15}\) per batch/lot of carcases of the same epidemiological unit should be collected.

Accounting for the bacteria prevalence, possible missingness and possible loss during storage, for the definition of the number of samples to be collected in each quarter of the year from each selected slaughterhouse should ensure that the isolates required by the monitoring programme are collected. If after the first quarter the CA realises that the number of samples is not sufficient to obtain the expected number of isolates, the number of samples to be collected in the following quarter(s) may be adapted and recalculated (see Scenario V).

5.3.1. Proportional allocation

Each MS will rank all slaughterhouses by throughput of eligible animals between 1 January and 31 December, in the previous year or according to the latest data available. Starting with the slaughterhouses of largest throughput, sufficient slaughterhouses should be enrolled to cover at least 60% of the national throughput of animals domestically produced. A list of participating slaughterhouses is then compiled, and the predetermined number of carcases to be sampled is distributed according to the proportional throughput from 1 January to 31 December in the previous year.

5.3.2. Simple random sampling of days, batch(es) of carcases and carcases

For each month, sampling sessions should be allocated randomly to the days of the month, based on operating days for that particular slaughterhouse. Simple random sampling techniques, as previously described, should be used. Preferably, a maximum of one caecal sample per slaughterhouse is sampled per day to ensure that there is no correlation between positive results that may derive from direct or indirect contact between sampled animals before slaughter. For this purpose, a number of sampling days (equal to the sample size) are randomly selected from the operating days of the slaughterhouse.

For each slaughterhouse each month, a number between 1 and 31 shall be selected at random. If the randomly selected number is a slaughtering day, for that month, then that day is selected for sampling. If not, then a new number is selected randomly. This process is preferentially performed once a month and repeated so many times as there are samples to be collected at the slaughterhouse. In addition, a number of practical issues may need to be taken into account when defining sampling days and samples to be taken at slaughterhouse. Sampling days may need to be restricted based on laboratory and courier limitations, e.g. in some situations, it may be necessary to exclude Fridays. For each sampling day, if more batches are slaughtered and more than one sample a day is collected, batches to be sampled should be selected randomly. From each selected batch/lot of carcases originating from the same epidemiological unit, one caecal content sample (single or pooled) from healthy\(^{16}\) animal(s) should be randomly selected and collected. This would ensure that only one isolate

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\(^{14}\) In the case statistics on the annual throughput of **domestically produced** meat of slaughterhouses are not available, statistics on the annual throughput of meat (whatever is the origin of the animals slaughtered) are used.

\(^{15}\) For broilers, a pooled caecal sample should be collected from the caecal content of 10 carcases randomly selected from the epidemiological unit. For turkeys, pigs and calves, some caecal content collected from one carcase randomly selected from the epidemiological unit should provide enough material to perform the requested testing.

\(^{16}\) Condemned or underweight carcasses and animals undergoing emergency slaughter should be notably excluded.
per epidemiological unit of origin per year is finally submitted to susceptibility testing. Where it is not possible to sample only one caecal sample per day per slaughterhouse due to logistical reasons, it may be proposed that up to five caecal samples per day can be sampled.\textsuperscript{17}

A stratification by slaughterhouse does not rule out the possibility of taking account of production types (e.g. for broilers: organic, free-range, standard), notably where relevant statistics/information are available.

6. Sampling designs of representative isolates from meat at retail

6.1. Objective

Samples of fresh meat collected at retail level, without differentiating between domestic and imported products, provide a better estimate of consumers’ exposure to resistant bacteria.

The objective is to collect at retail 300 representative random samples of fresh meat of broilers, turkeys, pigs and bovine animals, respectively, and to test them for the presence of ESBL-/AmpC-/carbapenemase-producing isolates of \textit{E. coli}. However, in MSs with a production of less than 100,000 tonnes of broiler meat slaughtered per year, less than 100,000 tonnes of turkey meat slaughtered per year, less than 100,000 tonnes of pig meat slaughtered per year and less than 50,000 tonnes of bovine meat slaughtered per year,\textsuperscript{18} the MS shall analyse 150 samples instead of 300 samples for each corresponding specific combination.

6.2. The delineation of the meat categories

The eligible categories of fresh meat of broilers, turkeys, pigs and bovine animals cover those marketed at the retail stage in the NUTS-3 area representing at least 80% of the population in the MS.

Within the framework of this sampling plan, retail is understood as outlets selling directly to the final consumer for subsequent domestic consumption, i.e. outlets such as supermarkets, specialist shops, markets and excluding catering activities, restaurants, wholesalers and similar outlets.

Within the framework of this sampling plan, fresh meat is understood as chilled meat\textsuperscript{19} (meaning that frozen meat is excluded), including meat that is wrapped, vacuum-wrapped or wrapped in a controlled atmosphere. As there is a specific interest as regards the consumer being exposed further up the food chain, then carcases or meat portions with skin on from broilers should be sampled as a measure of the likely heaviest degree of contamination from the animals. Selecting a standard type of meat that is readily available in all MS is important for harmonised monitoring. Un-skinned carcases/meat portions (diced) and skinned breast for broilers and samples of meat of pigs, and meat of cattle (both displayed without skin, typically) (sliced or diced) should be preferentially sampled.

6.2.1. Sampling design

A proportionate stratified sampling scheme is used at the MS level whereby the samples are allocated proportionally to the size of the human population in the regions (NUTS-3 area) accounting for at least 80\%\textsuperscript{18} of the national population. Next, at the second level, are the retail outlets to be sampled. At the third level, samples within the different meat categories to be sampled are selected. The 300/150 samples (of each meat category) are to be allocated in proportion to the size of the human population in the NUTS-3 area. The assumption underpinning the allocation of sample numbers to the regions, within MS, according to the size of their human populations, is that the human population sizes are fairly proportional to the volume sizes of the selected food categories on the market.

For the purpose of the proportional allocation of sample numbers, the following parameters are considered when adapting the generic stratified sampling approach to this specific sampling plan:

\textsuperscript{17} In exceptional cases, when it appears for particular economical and organisational reasons that it would be more cost-effective, a greater number of samples in a day could be collected, e.g. more than five caecal samples may be sampled per day per slaughterhouse, provided that a potentially introduced clustering effect is accounted for by increasing the sample size of the monitoring.

\textsuperscript{18} According to the most recent data available at EUROSTAT (http://epp.eurostat.ec.europa.eu).

\textsuperscript{19} In those MSs where there may be particular constraints on food distribution and where a substantial fraction of fresh meat is commercialised frozen at retail in certain regions, a complementary sampling of frozen fresh meat at retail may be performed. Corresponding data should be reported separately.
• **Strata**: the NUTS-3 area accounting for at least 80% of the national population\(^{20}\);
• **Stratum capacity**: the number of inhabitants according to the most recent data available;
• **Epidemiological unit (EpiU)**: lot of chilled fresh meat. No more than one sample per epidemiological unit per year should be collected.

Ideally, the central CA should draw up a sampling plan following the rules described below and based on the best marketing data available.

**Selection of the retail outlet categories to be targeted**

The CAs are responsible for choosing the retail outlets to be included. Typical types of retail outlets that could be included for sampling are: supermarkets and small shops/specialty delicatessens (e.g. traditional craft butcher’s businesses). Stratification on the major types of retail outlets allows for accounting for potential differences in supply chains (imported vs. domestic) and meat production types (e.g. for broiler meat: farmer’s, organic, free-range, standard etc.). The following rule shall be used to choose the types of retail outlets to be sampled and needs to be followed for each of meat categories:

- If the biggest category of outlets (e.g. supermarkets) supply at least 80% of the market of a meat category, then samples only need to be taken from those outlets. Where that is not the case, the second largest outlet category should be added and so on until at least 80% of the market is covered.
- The number of samples that should be taken from each retail outlet category included in the sampling plan should be proportionate to the market share of that outlet category within the targeted outlet categories.

If no major differences in supply chains and meat production types are expected between the categories of outlets, sampling may be limited to the biggest category of outlets in the MS to reduce logistical constraints.

With respect to the definition of sampling days and selection of the samples to be taken within each epidemiological unit, simple random sampling techniques should be used. In addition, a number of practical issues may need to be taken into account when defining sampling days and samples to be taken at retail. For each month sampling sessions should be allocated randomly to the days of the month, based on working days. Sampling days may need to be restricted based on laboratory and courier limitations, e.g. in some situations, it may be necessary to exclude Fridays.

At the same visit to a retail outlet, up to five different meat batches/lot per meat category can be sampled without preselecting samples based on the origin of the food. It is essential that cross contamination is avoided during the collection of fresh meat samples. Precautions must therefore be taken at all stages to ensure that the equipment used during sampling, transport and storage is not contaminated.

7. **Sampling designs of representative isolates from meat at BCPs**

7.1. **Objective**

The objective is to collect and test for antimicrobial susceptibility representative isolates of:

- *Salmonella* spp. from samples of fresh meat of broilers and turkeys,
- *E. coli* from samples of fresh meat of broilers, turkeys, pigs and bovine animals,
- Extended Spectrum Beta-Lactamase (ESBL)/-AmpC-/carbapenemase-producing *E. coli* from samples of fresh meat from broilers, turkeys, pigs and bovine animals, taken at border control points (BCPs).

The MSs assess which specific combinations of animal populations and bacteria should be sampled according to Decision 2020/1729, what number of isolates/samples should be collected and tested, and in which years. Fresh meat of broilers and turkeys should be sampled in 2022, 2024 and 2026, whereas fresh meat of pigs and bovine animals should be sampled in 2021, 2023, 2025 and 2027.

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\(^{20}\) A sampling frame representing at least 80% of the target population is proposed as a logistic compromise, since the legislation does not foresee any restriction of the study population. However, in certain MSs it might be necessary to further limit the NUTS-3 areas involved in the sampling frame to the regions with high population density to account for logistical and organisational constraints, in particular in those geographically large MSs with sparsely populated regions.
7.2. The delineation of the meat categories

The eligible categories of fresh meat of broilers, turkeys, pigs and bovine animals cover those consignments entering the customs territory of the EU at BCPs designated for fresh meat. All BCPs designated for fresh meat are included in the sampling plan. Within the framework of this sampling plan, fresh meat is understood as meat that has not undergone any preserving process other than chilling, freezing or quick-freezing, including meat that is vacuum-wrapped or wrapped in a controlled atmosphere. There is a specific interest as regards the consumer being exposed further up the food chain.

7.2.1. Sampling frame: representative consignments per BCP

Every EU Member States and every country concerned (Switzerland, Iceland and Norway) receiving consignments of fresh meat of broilers, turkeys, pigs and bovine animals from third countries to EU/EEA/EFTA is included in the sampling frame. The intention is to monitor AMR in zoonotic and indicator bacteria from fresh meat on a rotating basis: poultry meat in even years; pig and bovine meat in odd years.

A proportionate stratified sampling scheme is applied at the EU level whereby the samples are allocated proportionally to the number of consignments of fresh meat per BCP and per country of origin. Still, every BCP receiving at least one consignment is included within the country, the combinations of BCP and country of origin being considered the strata. A representative number of consignments arriving at the BCP from a country of origin is randomly selected to obtain samples of fresh meat; each consignment is considered a cluster. The number of samples to be taken is globally proportionate to the number of consignments per origin. Sampling design is stratified per BCP and origin (the combination is considered as strata).

In order to keep the sampling process manageable and considering that AMR is known to fluctuate across the year, the total number of consignments to be sampled must be evenly distributed along the year. It is of note that the sampling design requires a balance between a spatially and temporally representative sampling scheme, the total volume of samples, which can be effectively collected, processed and analysed, and the overall cost of the monitoring.

7.2.2. Sample size

Sample size calculation formulas are based on estimation of a parameter of interest (in this case, the proportion of consignments of fresh meat harbouring a resistant bacterium to a given antimicrobial imported in the EU). The rationale behind the calculation is to first fix the desired margin of error, defined as the error which the risk manager is willing to accept in estimating the parameter of interest. In order to ensure that the proposed sample size accounts for maximum variability, the value used for the expected proportion (\( \pi \)) for each type of meat is fixed at 0.5 (50%), which corresponds to a variability of 0.25 (\( \pi \cdot (1 - \pi) \)).

Sample sizes obtained are based on the consideration that the population size is infinite. Based on TRACES data available for 2017 and 2018, this assumption seems acceptable for broiler meat and bovine meat for which the number of consignments imported in the EU is large enough. For pig meat and turkey meat, the same assumption is likely to be acceptable in 2021 onwards. The number of samples to be taken for each type of fresh meats is based on the current information available, which consider that broiler and bovine meat population is infinite. As turkey and pig meat imports from the UK to EU are not yet reported in TRACES statistics as third countries import, and considering that the population size for those type of meats will increase after January 2021, it is then expected that the sample size will increase.

Using a simple random sampling scheme, the total sample size in the EU to be taken to achieve the accepted precision (margin of error) is given in Table 8 below. The accepted margins of error proposed are a trade-off between necessity and feasibility; they are not higher than that applied for monitoring ALMR within the EU.
7.2.3. Multistage sampling scheme and sampling fractions per type of meat

Simple random sampling ignores some of the complexities inherent in natural systems. In order to account for the large size of the consignments, multiple samples would need to be taken from the same consignment and they may be correlated. Where sampling units are not independent, the sample size can be obtained by adjusting the variance with the design effect. Multistage sampling designs use design effects to ensure that the correlation between sampling units from the same clusters are accounted for when calculating sample size. For consignments, the sampling unit is samples of meat, those could be correlated due to similarity between meat of a given consignment. The sample size calculation needs to account for this correlation. Ideally, correlation should be known prior to starting the monitoring. The correlation between samples in a given consignment used for this sample size calculation has been considered to be 0.1.

The final sample sizes can be calculated, considering the cluster size to be 3 (3 samples to be taken per consignment randomly selected) and the correlation to be 0.1. The resulting design effect will be 1.2, which should be further multiplied by the sample size (number of samples) to be sampled when considering the simple random sampling scheme (cf. Table 8) producing the total number of samples to be taken for consignments from BCP and origin. The number of consignments to be sampled per BCP and per origin is finally obtained by dividing the total number of samples to be taken by 3 (number of samples per consignments). It is also of note that collecting more than one sample per consignment randomly selected will reduce the number of consignments to be sampled compared with gathering only one sample per consignment, for a given proportion to be estimated with a given margin of error.

In accordance with the projections made based on 2017 and 2018 TRACES data, the corresponding indicative sampling fractions per type of meat and number of samples taken per consignment selected are presented in Table 9. All calculations were rounded up in order to ensure the level of precision required.

It is of note that the sampling design proposed is based on the reliable existing TRACES statistics, and the UK leaving the EU effect cannot be considered at this stage, because of the major uncertainties still associated with it. The technical specifications are indeed intended to highlight the current uncertainties related to Brexit, and the intention is to reassess the sampling design proposed in the light of the real trade flows observed in 2021, as reported through 2021 TRACES statistics, by the end of 2021.

### Table 8: Indicative total sample size for consignments to achieve the accepted precision

| Type of fresh meat | Accepted margin of error | Total sample size for consignments |
|-------------------|---------------------------|-----------------------------------|
| Broiler meat*     | 4%                        | 601                               |
| Turkey meat*      | 7%                        | 196                               |
| Pig meat**        | 5%                        | 236                               |
| Bovine meat**     | 4%                        | 601                               |

$: In estimating a proportion of 0.5 (50%).
*: Based on 2018 data.
**: Based on 2017 data.
It is also worth noting that the UK leaving the EU will most likely bring changes to trading arrangements and trade flows between the UK and the EU that will most likely need to be considered when TRACES data will become available. In particular, if the number of consignments arriving at the BCPs increases (notably due to the leaving of the UK from the EU), it is expected that the sampling fraction is going to decrease, but the total number of samples in principle will increase. As the sampling fractions and the number of samples to be taken per consignment are only indicative, MSs will also have the possibility to adapt them to their particular situation, in particular, regarding pig meat and turkey meat. If MSs have already detailed data available, MSs can consider them when designing the sampling plan. If the consignment population is considered similar to any of the presented ones above, then the design could be adapted accordingly.

The number of samples to be taken per consignment randomly selected could optimally also take into account the consignment’s weight for the important consignments only (e.g. with a weight greater than the average weight of consignments). For those consignments, the number of samples to be taken could be proportionate to the increase of weight compared with the average weight of the consignments of the same origin received in the MS/country in the previous year. If the weight of the consignment is greater than the average weight of consignment (year n – 1), the number of samples to be taken is proportional to the weight. This has not been considered in the table above based on projection made based on 2017 and 2018 TRACES data.

Eventually, there is a requirement to consider the possibility that during the monitoring, there may be a failure to take or analyse a sample. For this reason, it is good practice that the MSs/countries plan to slightly inflate the sample size to ensure the required precision is achieved. The inflation to account for possible missingness would be increasing the number of samples by a factor representing the expected percentage of missingness. This has not been considered in the table above based on projection made based on 2017 and 2018 TRACES data.

### Table 9: Indicative sampling fractions per type of meat and number of samples per consignment selected, based on 2017, 2018 TRACES statistics

| Type of fresh meat | Indicative sampling fraction per BCP and origin | Indicative number of samples taken per consignment |
|-------------------|-----------------------------------------------|--------------------------------------------------|
| Broiler meat*     | • up to 60 consignments arriving per BCP and origin: at least 1 consignment randomly selected sampled per year  
• above 60 consignments imported: an average of around 3% of consignments randomly selected sampled per year | 3 samples**** to be taken per consignment randomly selected |
| Turkey meat*      | • up to 10 consignments arriving per BCP and origin: at least 1 consignment randomly selected sampled  
• above 10 consignments imported: an average of around 15% of consignments randomly selected sampled per year | |
| Pig meat**        | • up to 10 consignments arriving per BCP and origin: 1 consignment randomly selected sampled  
• above 10 consignments imported: an average of around 15%*** of consignments randomly selected sampled per year | |
| Bovine meat**     | • up to 50 consignments arriving per BCP and origin: 1 consignment randomly selected sampled  
• above 50 consignments arriving per BCP and origin: an average of around 2% of consignments randomly selected sampled per year | |

*: Based on 2018 data.  
**: Based on 2017 data.  
***: A reduced sampling fraction of 10% can be accepted in the case of a higher number of consignments to be monitored.  
****: Flexibility regarding the number of samples to be taken per randomly selected consignment can be envisaged, given the remaining uncertainties associated with the impact of the leaving of the UK from the EU on trade flows.
7.2.4. Sampling design

First, a proportionate stratified sampling scheme is applied at the EU level whereby the samples are allocated proportionally to the number of consignments of fresh meat per BCP and per country of origin.

A sampling plan is to be designed by the CA at the beginning of the year in order to ensure that the required number of representative bacterial isolates is collected from each category of fresh meat and eventually submitted for susceptibility testing. For this purpose, the following parameters are considered:

- **Strata (1st stage):** all BCPs designated for fresh meat.
- **Stratum capacity:** the annual number of consignments arriving at the BCP during the previous year.
- **Strata (2nd stage):** For the purpose of this sampling at BCPs, consignments of meat are used for a second stage selection. The number of meat samples per consignment selected should be collected.

7.2.5. Systematic sampling

Each MS will rank all BCPs by the annual number of consignments of eligible meat categories and by country of origin between 1 January and 31 December, in the previous year or according to the latest data available. All BCPs per country should be enrolled in the sampling plan.

So that all BCPs per country are enrolled, the first consignment of each eligible meat category arrived from a given origin at each BCP should be sampled. To account for possible missingness and possible loss during storage, it may be desirable to plan to sample a second consignment randomly selected over the year. In practice, specific precautions have to be taken to collect samples from the earliest consignments not to fully miss a stratum (combination BCP/origin).

For those combinations of BCP and origin for which the number of consignments of either broiler meat, turkey meat, pig meat or bovine meat arrived in the previous year (or according to the latest data available) is lower or equal to 60, 10, 10, 50 consignments, respectively, at least one consignment randomly selected should be sampled per year. In practice, specific precautions have to be taken to collect samples from the earliest consignment not to fully miss a stratum (combination BCP/origin).

For those combinations of BCP and origin for which the number of consignments of either broiler meat, turkey meat, pig meat or bovine meat arrived in the previous year (or according to the latest data available) is greater than 60, 10, 10, 50 consignments, respectively, a systematic sampling should be implemented per BCP and origin as follows: 1 every 33 consignments of broiler meat, 1 every 7 consignments of turkey meat and pig meat and 1 every 50 consignments of bovine meat should be sampled. This sampling scheme will ensure that targeted number of samples is achieved at the end of the year, ensuring also the level of precision required to estimate the parameter of interest.

8. Review of the monitoring of AMR

It is recommended that these technical specifications be reassessed and updated regularly in the light of the results of the first monitoring campaigns and trends observed in the constantly emerging and evolving area of AMR. It is also worth noting that the UK leaving the EU will most likely bring changes to trading arrangements and trade flows between the UK and the EU that may need to be considered while revising sampling design and sampling fractions for monitoring AMR in bacteria from imported meat.

The sampling design proposed is based on the reliable existing TRACES statistics, and the effect of the UK leaving the EU cannot be considered at this stage, because of the major uncertainties still associated with it. These technical specifications highlight these uncertainties, and the intention is to reassess the sampling design proposed in the light of the real trade flows observed in 2021, as reported through 2021 TRACES statistics, by the end of 2021. The monitoring of AMR in imported meat is introduced in 2021, and the year 2021 will allow to gain experience and knowledge on this topic. As such, the year 2021 should be considered as a transition year regarding in particular this specific part of the harmonised monitoring of AMR.

In a number of the sampling plans proposed above, sampling frames may not represent the full target population (as a compromise at least 60% or 80% of the study population), provided that any particular subpopulations (e.g. those animals produced by particular operators or raised in particular
regions) are systematically excluded. It is, therefore, recommended that MSs assess how well the sampling frame matches the target population and whether any subpopulations are less represented to help understand any limitations in the monitoring programmes. An annual assessment of representativeness of each monitored population will enable EFSA and MS to better understand the outcomes of the monitoring. Changes to representativeness, over time or between MS, may help explain changes in AMR patterns or differences between MS.

A review and a regular update of these technical specifications would allow for the adjustment of sampling frames (stratifications), sample sizes and data collection procedures and would account for any improvement in laboratory methods incorporated into the programme and the most recent literature available. This would increase the quality of occurrence of resistance estimates in future monitoring.

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Glossary
For the purpose of this document, the following definitions apply.

**Border control post**
an inspection post designated and approved in line with EU legislation for carrying out checks on animals and animal products arriving from third countries at a European Union border. These checks are carried out to protect animal and public health, and animal welfare.

**Capacity**
total number of elements on which a stratum consists. For the purpose of this document, capacity is represented by: (i) the number of isolates available in the case of official laboratories; (ii) the number of animals slaughtered in the case of slaughterhouses; (iii) the size of the human population in the case of NUTS-3 regions.

**Competent authority**
means the central authority of a Member State competent for the organisation of official controls or any other authority to which that competence has been conferred, it shall also include, where appropriate, the corresponding authority of a third country.

**Domestically produced animals**
animals born and bred within the country of slaughter, and animals that spent part of their breeding life in the slaughter country, during which they may have been treated with antimicrobials. Domestically produced broilers, fattening turkeys may be defined as birds hatched and raised in the MS as well as those imported/traded as day-old chicks into the MS (i.e. hatched abroad). Domestically produced fattening pigs and bovine animals of less than 1 year of age may be defined as those animals produced and slaughtered in the MS and excluding those imported and intended for direct slaughter after importation. Therefore, pigs imported/traded into the MS as post-weaners, growers and fatteners are considered as domestically produced animals. As a general guideline, domestically produced animals should spend at least about 50% of their lifespan in the MS for the purpose of this document, it represents the unit of a population from which representative samples at primary production and slaughterhouse level should be collected; in particular, these are

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21 Regulation (EC) No 882/2004 of the European Parliament and of the Council of 29 April 2004 on official controls performed to ensure the verification of compliance with feed and food law, animal health and animal welfare rules, OJ L 165, 30.4.2004, p. 1.
represented by flocks (poultry) or slaughter batches (pigs and bovine animals) (see slaughter batch).

**Fresh meat**

meat that has not undergone any preserving process other than chilling, freezing or quick-freezing, including meat that is vacuum-wrapped or wrapped in a controlled atmosphere. It is of note that within the framework of the sampling plan of fresh meat at retail, fresh meat only covers chilled meat, including meat that is wrapped, vacuum-wrapped or wrapped in a controlled atmosphere.

**Harmonised monitoring of antimicrobial resistance**

monitoring aimed at providing comparable data on the occurrence of antimicrobial resistance in zoonotic and commensal bacteria.

**Isolate**

microorganism obtained in pure culture from a sample.

**Isolation**

'any procedure in which a given species of organism, present in a particular sample or environment, is obtained in pure culture’ (Singleton and Sainsbury, 1987).

**Monitoring**

system of collecting, analysing and disseminating data on the occurrence of zoonoses, zoonotic agents and antimicrobial resistance related thereto (Directive 2003/99/EC) – conducting a planned sequence of observations or measurements with a view to obtaining an overview of the state of compliance with feed or food law, animal health and animal welfare rules.

**NUTS-3 area**

The Nomenclature of Territorial Units for Statistics (in French: Nomenclature des unités territoriales statistiques) (NUTS) is a geocode standard for referencing the subdivisions of countries for statistical purposes. The NUTS regions are based on the existing national administrative subdivisions and there are three levels of NUTS defined. The current NUTS classification, valid from 1 January 2012 until 31 December 2014, lists 97 regions at NUTS-1, 270 regions at NUTS-2 and 1294 regions at NUTS-3 level. This category refers to regions belonging to the third level (NUTS-3), which is largely used by EUROSTAT and other European Union bodies. Depending on the size of the country, the NUTS-3 level may be a region, a county, a department, a group of municipalities etc.

**Random sample**

It is a sample which is taken under statistical consideration to provide representative data. Random sampling is used to obtain a sample whose results can infer to the wider population. It is used to maximise external or ecological validity.

**Retail**

the handling and/or processing of food and its storage at the point of sale or delivery to the final consumer. In this document, retail covers only shops, supermarkets and other similar outlets that sell directly to the final consumer. It does not include distribution terminals or centres, factory canteens, restaurants and other similar food service operations and wholesale outlets.

**Sample**

'A subset of a population selected for inclusion in a study' (Toma et al., 1999). In the framework of this document, samples from different matrices are collected and submitted to isolation of specific bacteria.

**Sampling**

'The process of selecting elements from a population in order to construct a subset (sample) to be used for making inferences about the population' (Toma et al., 1999).

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22 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/EEC. OJ L 325, 12.12.2003, p. 31–40.

23 Commission Decision 98/179/EC of 23 February 1998 laying down detailed rules on official sampling for the monitoring of certain substances and residues thereof in live animals and animal products, OJ L 65, 5.3.1998, p. 31–34.

24 Regulation (EC) No 178/2002 of the European Parliament and of the Council of 28 January 2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety, OJ L 31, 1.2.2002, p. 1–24.
**Sampling frame**

‘the assemblage of individuals (or groups) on which sampling is performed’ (Toma et al., 1999)

**Sampling frame complete list of all units of the population which can be sampled**

**Sample size**

the number of units randomly chosen from the sampling frame

**Slaughter batch**

for the purpose of this document, means a group of animals originating from the same herd, raised together under the same conditions and sent to the slaughterhouse on the same day, and therefore, representing a batch/lot of carcases produced from animals slaughtered on the same day and originating from the same flock of broilers/fattening turkeys or the same (fattening/growing) batch of fattening pigs/bovine animals under 1 year of age

**Stratum/strata**

‘Two or more subsets of a study population defined by mutually exclusive characteristics’ (Toma et al., 1999). For the purpose of this document, strata may be defined depending on the level at which sampling or collection of isolates is to be performed: i) in the case of isolates originating from samples collected at primary level, strata are represented by laboratories with available collections of isolates (considered separately per animal population of study); ii) in the case of isolates originating from samples collected (carcase samples) or to be collected (caecal samples) at slaughterhouse level, strata are represented by slaughterhouses; iii) in the case of isolates originating from samples to be collected at retail level, strata are represented by NUTS-3 regions; iv) in the case of isolates originating from samples to be collected at BCPs, strata are represented by the combinations of BCP and the country of origin.

**Abbreviations**

- **AMR** Antimicrobial resistance
- **BCP** Border control point
- **CA** Competent Authority
- **EEA** European Economic Area
- **EFTA** European Free Trade Association
- **EpiU** Epidemiological unit
- **ESBL** Extended Spectrum Beta-Lactamase
- **FBO** Food Business Operator
- **MS** Member State
- **NCA** National Competent Authority
- **NCP** National Control Programme
- **NUTS** Nomenclature of territorial units for statistics
- **PCR** Polymerase chain reaction
- **spp.** species
- **SRS** Simple random sampling
- **TRACES** The European Commission’s multilingual online platform for sanitary and phytosanitary certification required for the importation of animals, animal products, food and feed of non-animal origin and plants into the European Union, and the intra-EU trade and EU exports of animals and certain animal products