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TECHNICAL REPORT

Manual for reporting on antimicrobial resistance within the framework of Directive 2003/99/EC and indicator bacteria for information derived from the year 2013[1]

European Food Safety Authority[2, 3]

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ABSTRACT

This Reporting Manual provides guidance for reporting on antimicrobial resistance under the framework of Directive 2003/99/EC as well as indicator bacteria in animals, food and feed. Also guidance for reporting on prevalence, genetic diversity and antimicrobial resistance in methicillin-resistant *Staphylococcus aureus* (MRSA) from food-producing animals, and food derived thereof, is included. The objective is to harmonise and streamline the reporting made by the Member States to ensure that the data collected are relevant and easy to analyse at the European Union level. Detailed guidelines are provided for reporting of data in the table and text forms. This guidance typically applies to *Salmonella*, *Campylobacter coli* and *jejuni*, animal species and food categories to be reported on. Guidance is also provided on indicator *Escherichia coli* and *Enterococcus* and methicillin-resistant *Staphylococcus aureus* (MRSA).This manual is specifically aimed at guiding the reporting of information deriving from the year 2013.

KEY WORDS

reporting, antimicrobial resistance, zoonotic bacteria, indicator bacteria, MRSA

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SUMMARY

This Reporting Manual provides guidance on reporting on antimicrobial resistance under the framework of Directive 2003/99/EC as well as indicator bacteria in animals, food and feed. Also guidance for reporting on prevalence, genetic diversity and antimicrobial resistance in methicillin-resistant *Staphylococcus aureus* (MRSA) from food-producing animals, and food derived thereof, is included. The objective is to harmonise and streamline the reporting made by the Member States to ensure that the data collected are relevant and easy to analyse at the European Union level. Detailed guidelines are provided for reporting of data in the table and text forms through the web-based application. This manual typically applies to the agents, animal species and food categories to be reported. Instructions are given on the description of the sampling and monitoring schemes as well as analyses of the results in the national reports.

This manual covers *Salmonella*, *Campylobacter coli* and *jejuni*, indicator *Escherichia coli*, *Enterococcus* and methicillin-resistant *Staphylococcus aureus* (MRSA) included in the current data collection through the web-based reporting system. These instructions are also applicable to reporting through the Data Collection Framework. Detailed guidelines are provided on reporting of the data in the table and text forms of the web reporting application.

This manual is specifically aimed at Member State data providers to guide the reporting of information deriving from the year 2013.

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BACKGROUND AS PROVIDED BY EFSA

The Directive 2003/99/EC lays down the European Union (EU) system for monitoring and reporting of information on zoonoses, which obligates the Member States to collect data on zoonoses, zoonotic agents, antimicrobial resistance and food-borne outbreaks. EFSA is assigned the tasks of examining the data collected and preparing the EU Summary Reports in collaboration with the European Centre for Disease Prevention and Control (ECDC).

Based on the data reported each year, EFSA and ECDC will jointly produce an annual EU Summary Report on zoonoses, zoonotic agents and food-borne outbreaks. Similarly, the two agencies will produce a EU Summary Report on antimicrobial resistance. To support the Member States in their reporting, the existing reporting manuals for zoonoses, antimicrobial resistance and food-borne outbreaks need to be updated to take into account the latest recommendations on reporting of antimicrobial resistance data and data on zoonoses and food-borne outbreaks. In addition, the manuals have to be revised due to the changed structure of the reporting tables in the web application and changes in the relevant EU legislation.

EFSA runs a web-based reporting application for the annual reporting as well as the possibility of submitting data in XML/Excel format via the Data Collection Framework (DCF). The amended web application has to be tested and new XML reporting schemas created before the start of the reporting period in April each year. This is supported by revised guidance documents.

For quality improvement purposes, the EU Summary reports will be submitted every third year to the Scientific panels of Biological Hazards and Animal Health and Welfare for their review and comment.

In addition to these EU Summary Reports, EFSA will, in collaboration with ECDC and the European Medicine Agency (EMA), prepare, in the future, joint reports combining antimicrobial resistance and consumption data. These reports will be separate from the EU Summary reports and are covered by another mandate (M-2012-0209).

TERMS OF REFERENCE AS PROVIDED BY EFSA[4]

The BIOCONTAM and DATA units[5] are invited to:

- prepare and publish the EU Summary Reports on Zoonoses, Zoonotic agents and Food-borne Outbreaks in 2012, 2013 and 2014 in close collaboration with ECDC;
- prepare and publish the EU Summary Report on Antimicrobial Resistance in 2012, 2013 and 2014 in close collaboration with ECDC;
- revise the manual for reporting on zoonoses, zoonotic agents and antimicrobial resistance each year, and publish it as an EFSA technical report;
- revise the manual for reporting on food-borne outbreaks when appropriate, and publish it as an EFSA technical report;
- to revise the user manual for the web reporting application each year and publish it as an EFSA technical report;
- to revise the guidelines (data dictionaries) for XML/Excel data reporting each year and publish them as an EFSA technical report.

This technical report specifically addresses the third term of reference above: revise the manual for reporting on zoonoses, zoonotic agents and antimicrobial resistance each year, and publish it as an EFSA technical report.

[4] Available online: http://registerofquestions.efsa.europa.eu/roqFrontend/questionsListLoader?mandate=M-2013-0063
[5] Since 1st January 2014, these tasks were moved from former BIOMO unit to the new BIOCONTAM and DATA units.

1. Introduction

1.1. Monitoring of zoonoses, antimicrobial resistance and food-borne outbreaks

The European Union (EU) system for monitoring and collecting information on antimicrobial resistance in food-producing animals, and food thereof, is established by Directive 2003/99/EC[6] on the monitoring of zoonoses and zoonotic agents. This Directive requires Member States (MSs) to collect, evaluate and report data on zoonoses, zoonotic agents, antimicrobial resistance and food-borne outbreaks to the European Commission (EC) each year. The system is based on those of the MSs, and in a few cases, it is harmonised by EC legislation to the extent that the results from the monitoring are comparable between the MSs. The MSs are required to send their national reports on antimicrobial resistance to the EC each year by 31st May. The EC shall submit this information to the European Food Safety Authority (EFSA), which shall examine the data and publish the EU Summary Report from the results.

It should be noted that data on antimicrobial resistance in *Salmonella* and *Campylobacter* isolated from human cases of salmonellosis and campylobacterisosis are provided through the EU network for the epidemiological surveillance and control of communicable diseases established under Decision No 2119/98/EC[7]. The EU Summary Report is prepared in collaboration with the European Centre for Disease Prevention and Control (ECDC).

1.2. Web-based reporting system

The provisions laid down in EU legislation have been retained in the current technical specifications (EFSA, 2007, 2008) issued by EFSA for the monitoring of antimicrobial resistance (AMR) in *Salmonella* and *Campylobacter* and indicator *E. coli* and enterococci, where the minimum requirements of Directive 2003/99/EC have been expanded to a full description of the elements to be included in both qualitative and quantitative tables for the reporting of aggregated data. These elements have also been implemented in the EFSA web-based reporting system which has been developed and is being used for the electronic transmission of the data from the MSs to EFSA. The data are submitted in the format of tables for each given combination of bacterial species/study population (animal or food category).

In the qualitative AMR tables, for each antimicrobial tested, the following information is reported: the number of isolates tested, the number of resistant isolates, the number of fully-susceptible isolates and the number of isolates resistant to 1, 2, 3, 4 or > 4 antimicrobials. In the quantitative AMR tables, for each antimicrobial tested, the relative minimum inhibitory concentration (MIC) distributions are reported, as the number of inhibited isolates at the corresponding values of antimicrobial concentration. For data obtained through the diffusion method, the different inhibition zone diameters are reported.

EFSA has established a web-based reporting system and database to streamline and harmonise the reporting of AMR data under Directive 2003/99/EC. This system shall be used for the purpose of reporting and it is accessible on the following website: http://www.efsa.europa.eu/zoonoses.

[6] 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.

[7] Decision No 2119/98/EC of the European Parliament and of the Council of 24 September 1998 setting up a network for the epidemiological surveillance and control of communicable diseases in the Community. OJ L 268, 3.10.1998, p. 1–7.

1.3. Reporting through the Data Collection Framework (DCF)

As an alternative to the web-based reporting, AMR data can also be submitted to the Data Collection Framework (DCF) using XML or Excel or CSV formats. Separate guidelines are given on technical details for this reporting system. Although there are some differences between the two reporting systems, similar principles are applied. Thus, this manual can also be used as guidance in reporting via the DCF.

Reporting isolate based AMR data

Information on multi-drug resistance (MDR) is not fully accessible due to the fact that aggregated data currently are mostly reported by MSs. AMR may occur in association, meaning that an isolate may be resistant to different classes of antimicrobials simultaneously. Many patterns of MDR may be encountered within the same bacterial subtype (e.g. serovar/serotype/phagetype and biotype). Analyses on MDR, specific co-resistance patterns and association between resistance traits cannot be performed on the currently available dataset deriving from aggregated data. In order to perform such analyses information needs to be collected with a greater level of granularity, and data must be reported at the isolate level tested for antimicrobial susceptibility.

The collection and reporting of AMR data at the isolate level enables more in-depth scientific analysis. In particular, it would be beneficial for detecting new MDR patterns and performing analysis of the known co-resistance ones, evaluating geographical progression over time, conducting retrospective analysis and assisting in source attribution. In addition, the evaluation of phenotypic resistance patterns can give insight into resistance selection, since use of one antimicrobial can select for resistance to other unrelated antimicrobials (co-selection). Therefore, the collection of data on multi-resistance is of the utmost importance for investigating the relationship between antimicrobial use and resistance.

It is also expected that submission of data at the isolate level would facilitate the reporting of detailed epidemiological information, such as the serovar of the *Salmonella* strains, the geographical area and production type/food category of origin. This should also ensure consistency with the detailed recommendations issued by EFSA (EFSA, 2012a) as regards the way data are presented in the EU Summary Report on AMR.

Given the public health relevance of the emergence of multi-resistant bacteria, it is strongly recommended that AMR data collection is performed at the isolate level. Moreover, it should be noted that, in the case of a switch to reporting at isolate-based level, submission of both quantitative and qualitative data at aggregated level would become redundant since it would not provide any information in addition to that obtainable through the isolate-based data.

2. General guidelines for reporting

2.1. Structure of the zoonoses web-based reporting system

For each reporting year, a national report is created in the web-based reporting system. For each zoonoses or other subject, text forms and reporting tables are provided. The text forms are used to enter the narrative part of the report, e.g. description of the monitoring system and the analyses of the results. The reporting tables are used to enter the results, e.g. number of samples tested and number of positive results. Detailed instructions on how to use the text forms and reporting tables as well as the entire web application are given in the user manuals on the web-based reporting system homepage (http://www.efsa.europa.eu/zoonoses).

Mandatory reporting of AMR data

In accordance with the Zoonoses Directive 2003/99/EC, all MSs are required to report antimicrobial resistance in *Salmonella* and *Campylobacter* isolates from poultry, pigs and cattle and foodstuffs derived from these species. The requirements for the monitoring of antimicrobial resistance by the MSs are laid down in Directive 2003/99/EC. In particular, as regards the information that must be collected by the MSs, the following categories are listed in Annex II to the Directive:

1. animal species included in monitoring;
2. bacterial species and/or strains included in monitoring;
3. sampling strategy used in monitoring;
4. antimicrobials included in monitoring;
5. laboratory methodology used for the detection of resistance;
6. laboratory methodology used for the identification of microbial isolates;
7. methods used for the collection of the data.

The necessary AMR quantitative tables and text forms can be created in the web application by using the 'Report structure' tool or by reporting information on them to the DCF.

2.2. General guidelines on reporting the results in MRSA tables

General recommendations

The results (data) of investigations are reported in tables provided in the web-based reporting system or through the DCF. The types of data which are reported in the tables are mostly numerical, but also text type information can be requested for certain table cells.

The methicillin-resistant *Staphylococcus aureus* (MRSA) tables for animals and food have options for adding **additional *spa*-types**. Comments may be added to each reporting row, to provide further information on the specific investigation. In addition, general comments referring to the entire table can be added using the footnote. When no data are available, no value should be entered in the tables, not even the zero ('0') value. The zero value '0' may only be entered in instances of true zero results, e.g. no positive results from a number of units tested.

In case there is no relevant information to be reported or if the MS wishes not to report any data, the table should be left empty and marked as complete (see the user manual for the web-based reporting system) in order to indicate that no data will be submitted. However, when no positive units have been detected out of the units tested in the context of the investigations, a '0' (zero) should always be inserted in the column 'Total units positive for *Staphylococcus*' to indicate the testing results.

In the following zoonoses/agent sections the animal species/food categories particularly recommended to be reported are indicated by bold text.

Information requested in the rows

In the rows, data on foodstuffs (definitions are presented in Appendix D) and animals (definitions are presented in Appendix E) should be categorised using the classification system provided by the pick lists. There will be variability in the degree of detail which can be provided. However, reporters are strongly encouraged to provide as much relevant information as possible within the limits of the system. The reason is that the information provided by the pick lists enables relevant epidemiological data analyses.

MSs are asked to avoid double reporting into different category levels, i.e. data reported both in the total and in the detailed categories.

MSs are invited to report all relevant information on the type of animals or food sampled including the sampling stage and the sampling context (definitions are presented in Appendix C), when appropriate.

This information may include:

- The type of animal population sampled e.g. wild/farmed/zoo animals/pet animals for those populations that could fall under more than one typology, e.g. wild boar;
- The stage along the food chain where samples have been collected.

Information requested in the columns

Information that could be reported in the table columns (such as agent species or information on the sampling stage and context) should not be reported in the comments or footnote in order facilitate data extraction.

The total number of samples positive for ***S. aureus*, methicillin resistant (MRSA)** must equal the sum of the reported numbers of **spa-type** in their specific columns including the unspecified category column. An exception is the case where more than one species/serotype/serovars are isolated from the same sample. In this case, this fact should be stated in the comment adjacent to the reporting row.

2.3. General guidelines on reporting the antimicrobial susceptibility results in the tables

Antimicrobial susceptibility tables are provided for *Salmonella* and *Campylobacter*, as well as for *E. coli* and *Enterococcus* indicators as related to foodstuffs, animals and feedingstuffs. There are breakpoint tables and quantitative and qualitative antimicrobial susceptibility tables.

Cut-off value tables for antimicrobial resistance

The cut-off tables are provided to report cut-off values used in the antimicrobial susceptibility testing. The information to be reported is:

- **Test method used** - specify the test used: disc diffusion method, agar dilution, broth dilution or E-test®;
- **Standards used for testing** - specify whether the methods used for testing were defined according to National Committee for Clinical Laboratory Standards (NCCLS)/Clinical and Laboratory Standards Institute (CLSI) standards or according to other standards;
- **The methods used for investigation of isolates** - information entered in the table can be copied to other cut-off value tables under the same zoonosis section by clicking the relevant categories (food, animals and feedingstuffs) in the box 'The methods are used for investigation of isolates from'. The information will then be copied to the relevant table;

- **Standard** - specify which standard for cut-off value has been used for each susceptibility category (e.g. Decision 2007/407/EC,European Committee on Antimicrobial Susceptibility (EUCAST), EFSA's recommendations (EFSA 2007, 2008, 2012b,c), NCCLS);
- **Concentration (microg/ml)/Resistant >** (Dilution method) - is used to report broth and agar dilution cut-off values;
- **Zone diameter (mm) /Resistant <=** (Diffusion method) - is used to report disc diffusion cut-off values.

Please note that when creating a new annual report, reporting officers are able to choose the possibility of automatically importing, into the cut-off value tables, the cut-off values specified in the EFSA's recommendation 'Report of the Task Force on Zoonoses Data Collection including a proposal for a harmonized monitoring scheme of antimicrobial resistance in *Salmonella* in fowl (*Gallus gallus*), turkeys, and pigs and *Campylobacter jejuni* and *C. coli* in broilers' (EFSA, 2007) and in the EFSA's recommendation 'Report from the Task Force on Zoonoses Data Collection including guidance for harmonized monitoring and reporting of antimicrobial resistance in commensal *Escherichia coli* and *Enterococcus* spp. from food animals' (EFSA, 2008).

The cut-off values specified in these EFSA recommendations are imported automatically into the cut-off value tables for dilution methods for isolates from animals, food and feedingstuffs. Two different cut-off value tables are available for *C. jejuni* and *C. coli* as well as for *E. faecalis* and *E. faecium*.

Please note that it is not possible to use the automatic import function of the cut-off values once the annual report has been already created.

Please note that reporting officers can copy the cut-off value table information for the other sectors (food, animals, humans).

2.3.1. Quantitative antimicrobial resistance tables

These tables are used to report quantitative results from testing of bacterial isolates for antimicrobial resistance. The results are reported as the number of isolates with the given concentration/inhibition zone.

Please note that due to the automatic calculations made by the web application, the cut-off value table has to be filled in first to allow the calculations of the values of 'N' and 'n' in the quantitative tables. The cut-off values can be still manually changed in the quantitative tables.

The information that should be reported includes:

- **Isolates out of a monitoring programme (yes/no)** - indicate whether the isolates in the table originate from a monitoring programme or not;
- **Number of isolates available in the laboratory** - report the total number of isolates available in the laboratory testing for AMR, for a given reporting year, for the specific bacterial species or serovars and as far as possible broken down per animal population (e.g. laying hens or broilers) or food category. The reporting should be made at the bacterial species level for *C. jejuni*, *C. coli*, *E. coli* and *E. faecium* and *E. faecalis.* Regarding *Salmonella*, it is recommended to report at the serovar level if the isolates available have been serotyped. Regarding MRSA, it is recommended to report at the spa-type/MLST/clonal complex level, if the isolates available have been characterised. Please note that the same number for all isolates coming from the same monitoring context should be reported.
- **Column 'N'** - this column refers the number of isolates tested for susceptibility vis-à-vis the antimicrobial mentioned in the row heading; it is calculated automatically;
- **Column 'n'** - this column refers the number of resistant isolates (out of the N isolates tested) and is calculated automatically on the basis of the information provided concerning the corresponding cut-off values;

- **Dilution method (concentration (mg/L)), number of isolates with a concentration of inhibition equal to** - in every one of these cells, report the number of isolates with the concentration (mg/L) of inhibition equal to the column heading figure;
- **Agar diffusion method (Zone diameter (mm)), number of isolates with a zone of inhibition equal to** - in every one of these cells, report the number of isolates with the diameter (mm) of inhibition equal to the column heading figure.

2.3.2. Qualitative antimicrobial resistance tables

The qualitative tables are used to summarise the number of resistant strains for each antimicrobial substance for different food, animal species and feedingstuff categories. The number of multiresistant isolates is also reported in this table.

The Antimicrobial Qualitative Tables should be created by specifying first the food, feed or animal category and then, within the table, the agent serovars/species.

Specific guidelines for reporting data in the qualitative table:

- **Isolates out of a monitoring programme (yes/no)** - indicate whether the isolates in the table originate from a monitoring programme or not;
- **Number of isolates available in the laboratory** - report the total number of isolates available in the laboratory testing for AMR, for a given reporting year, for the specific bacterial species or serovars and as far as possible broken down per animal population (e.g. laying hens or broilers) or food category. The reporting should be made at the bacterial species level for *C. jejuni*, *C. coli*, *E. coli* and *E. faecium* and *E. faecalis.* Regarding *Salmonella*, it is recommended to report at the serovar level if the isolates available have been serotyped. Regarding MRSA, it is recommended to report at the spa-type/MLST/clonal complex level, if the isolates available have been characterised. Please note that you have to report the same number for all isolates coming from the same monitoring context.
- **Column 'N'** - number of isolates that are tested for susceptibility vis-à-vis the antimicrobial mentioned in the row heading, and for the animal species mentioned in column heading;
- **Column 'n'** - number of resistant isolates;
- For the rows **'Fully sensitive'** and **'Resistant to 1 to >4 antimicrobials'** the number of isolates tested should be reported in the **'N'** column and in the **'n'** column the number of isolates found fully sensitive or resistant to the specified number of antimicrobials should be indicated.

Please note that due to a function (ad hoc button) in the web reporting application, data providers can choose the possibility to automatically import the values entered in the Antimicrobial Quantitative Tables into the Antimicrobial Qualitative Table. If this option is chosen, the sum of the values reported in the corresponding Antimicrobial Quantitative tables, both for diffusion and dilution method, will be copied into the Antimicrobial Qualitative Table.
In case the data to be reported in the qualitative tables are exactly the same as those reported in the quantitative tables, there is no need to fill in the qualitative tables!

2.4. General guidelines on reporting the narrative part in the text forms

The narrative part should include a description of the monitoring and/or control programme from which the data are derived. This information ensures understanding and interpreting the results in the correct framework. The description should be detailed enough to give an accurate picture of the monitoring and control activities in place and to facilitate, where possible, the comparison of the results between reporting years.

In addition, an analysis of the results should be provided in the narrative part. This analysis may cover comparison of current results with those from previous years, in order to identify the trend. The

sources of zoonotic agents should be evaluated, particularly in relation to the relevance of the findings of zoonotic agents in foodstuffs, animals and feedingstuffs to human zoonoses cases.

For reporting the narrative part of the report, the text forms provided in the web-based reporting system and in DCF should be used. The information is entered in the text fields with the titles listed below.

The information below is recommended to be given under each title.

2.4.1. Monitoring system

Sampling strategy - this part describes, in general, the sampling strategy chosen and the purpose of the sampling:

- It is useful to state if the sampling covered the whole MS or only parts of it;
- The target population should be identified. It should be explained, for example, whether the entire animal population was covered or only a subset of it and the reasons for choosing this subset for sampling. Similarly the categories of foodstuffs and feedingstuffs that were sampled should be identified;
- If the sampling is stratified, for example, by geographical regions or other criteria, such as size of the holdings, this should be described;
- It is important to explain how the units to be sampled are chosen, regardless of whether objective, selective, suspected, convenience or census sampling is applied or if several sampling methods are applied;
- Reporters, reporting officers, data providers should specify who is performing the sampling, e.g. samples taken by the competent authority as part of an official sampling, samples taken by owners of animals, food or feed businesses, or by other representatives of private enterprises, in the context of HACCP/own checks;
- It is also essential to explain where the samples are taken, e.g. at farm, at slaughterhouse, at hatchery, at a food processing plant or at retail. Equally important is the stage of sampling, which can be any step in animal rearing process or the food chain. For example, the sample may be taken at animal rearing period, production period, before or after chilling of carcase in the slaughterhouses, before or after the expiration of the shelf-life of foodstuffs;
- The framework of the sampling is an important part of the strategy. It should be stated if the sampling is part of a permanent or temporary monitoring programme, linked to surveillance or control programmes or if it is the result of a single survey.

Frequency of the sampling - this part is intended to explain how often samples are taken. The standard terms (e.g. every week, once a month, x times a year) provided in the pick list in the text forms should be used where possible. A more general statement can also be used, such as 'Detection of annual prevalence of xx by yy % confidence level and zz % accuracy'.

Type of specimen taken - under this title, the specimen taken from the units sampled is described. For example, in the case of animals the specimen which is tested could be faeces, blood, organs or milk.

Methods of sampling (description of sampling techniques) - the sampling techniques, meaning the procedures on how the sample is technically taken, are described. This should include information on the site of sampling (e.g. part of a carcase, part of the facilities for environmental sample), size of sample taken (e.g. in g, cm^2, ml), use of swabs or other instruments in the sampling, where relevant, the number of (sub) samples/sample units taken, pooling of samples when conducted (refer the number of samples combined by pooling, if available), the possible storage of samples and the length of storage, where relevant.

Case definition/definition of a positive finding - this covers the description of when the sample is considered to be positive for the zoonotic agent or when the animal, herd or flock is considered to be

infected with the zoonotic agent. Regarding food and feed, it should describe when the foodstuff, feedingstuff or the batch sampled is considered to be positive or contaminated with the zoonotic agent.

Diagnostic/analytical methods used - under this title, the diagnostic or analytical methods used in the laboratory to test the specimens are described. Whenever possible, a reference to standard methods used is made (such as national, ISO or EN standard methods), or to the methods prescribed by the legislation. The year of reference of the method should be included. If these methods have been modified, the modifications made should be indicated to enable the comparison of the methods. It is also important to describe the quality assurance procedures in place in the laboratories. In addition, the procedure to prepare the sample in the laboratory should be described if it is relevant to the results. Section 4 provides more detailed information on how to describe an analytical method.

Other preventive measures than vaccination in place - preventive measures may include actions taken at different levels of the food chain. Regarding animals, it may cover, for example, consistent monitoring of potential livestock-associated MRSA reservoirs or bio-security measures at the farms. For the foodstuffs, it may include, for example, prohibition to market unpasteurised milk and recommendations on food consumption for susceptible consumer groups.

2.4.2. Control programmes/mechanisms

The control programmes/strategies in place - under this title, the control programmes in place in the MS should be described. The control programmes may be national or regional, and they may be approved nationally or by the Commission and co-financed by the EU based on Council Decision 2009/470/EC on expenditure in the veterinary field[8]. Control programmes run by the industry/food business operators are also included. The nature of the control programmes should be described including whether the programme is e.g. voluntary or mandatory, national or regional, approved by the EU or at national level or co-financed. The main features of the programme are given. It is advisable to report separately the information derived from official programmes and from programmes run by the industry. Other control mechanisms may include control measures prescribed in the EU or national legislation, such as rejection of contaminated carcases during meat inspection. The relevant legislation should be mentioned.

Measures in case of the positive findings or single cases - actions required by the legislation or control programmes as a consequence of positive findings in animals, foodstuffs or feedingstuffs should be explained. These measures may cover withdrawal of the products from the market, destruction of animals and others.

Notification system in place - the notification system is described, including its legal basis and since when the disease or infection has been notified.

Recent actions taken to control the zoonoses - specific measures undertaken during recent years to contain zoonoses are described. In the case of measures initiated in previous years, the year in which measures started to be applied should be indicated. These actions may include new legislation, recommendations issued, new control programmes, etc.

Suggestions to the EU for the actions to be taken - this item provides an opportunity to propose measures to be taken by risk managers at EU level. Typically, this could involve suggestions for new EU legislation.

2.4.3. Results of the investigation

The results reported and presented in the reporting tables are summarised. The important findings and the relevant conclusions based on the results should be presented.

[8] Council Decision 2009/470/EC of 25 May 2009 on expenditure in the veterinary field. OJ L 155, 18.6.2009, p. 30–45.

National evaluation of the recent situation, the trends and sources of infection - under this title, the results are interpreted in relation to their importance to public health in the MS. It is essential to evaluate the trend when compared to the previous year, e.g. when there is a decreasing or increasing trend or if the situation is stabilized. The important sources of infections should also be discussed.

Relevance of the findings in feedingstuffs/animals/foodstuffs and to human cases (as a source of infection) - in light of the results reported, the importance of feedingstuffs/animals/foodstuffs as sources of human infections should be evaluated. The role of feedingstuffs as a source of infection for animals, and similarly the role of animals as a source of contamination for foodstuffs should also be considered.

History of the disease and/or infection in the country - the history of the zoonoses cases in humans and animals in the past should be reported under this title. For example, the number of cases in the past and the impact of control and eradication programmes can be addressed.

Additional information - under this title, any other information relevant to the monitoring of the zoonoses in question can be given.

3. Specific guidelines for reporting

3.1. Methicillin-resistant *Staphylococcus aureus* (MRSA)

3.1.1. Methicillin-resistant *Staphylococcus aureus* in animals

Table 1: Recommendations on the food-producing animal populations and samples to collect for the MRSA monitoring

Animal populations	MRSA	
	Where to collect	Samples to collect
Monitoring recommended to be performed consistently on a regular basis (every third year)		
Broilers	Farm	Boot swab[(a)]
Fattening pigs	Slaughterhouse/Farm	Pool of nostril swabs[(b)]/Boot swab[(c)]
Dairy cattle	Dairy farm	Bulk tank milk
Monitoring recommended to be performed consistently on a regular basis, if production exceeds 10 million tonnes slaughtered/year (every third year)		
Fattening veal calves (under 1 year of age)[(e)]	Slaughterhouse	Nostril swabs
Fattening turkeys	Farm	Boot swabs[(a)]
Monitoring recommended to be performed on a voluntary basis (every third year)		
Breeders of pigs	Farm	Nose swab
Breeders of *Gallus gallus*, meat sector	Farm	Boot swab/Nose skin swab[(a)(d)]
Breeders turkeys	Farm	Boot swab/Nose skin swab[(a)(d)]
Beef animals	Slaughterhouse	Nostril swabs
Horses	Slaughterhouse	Nostril swabs

(a): In the framework of the *Salmonella* National Control Programmes, an additional boot swab sample may be obtained for MRSA testing.
(b): Sampling on farm is preferred for the purpose of assessing the risk factors for MRSA infection. In this case, larger pools of nose swabs can be collected.
(c): Sampling at slaughter or on farm depending on the considerations developed in the Technical specifications for the harmonised monitoring and reporting of antimicrobial resistance in MRSA in food-producing animals and food (EFSA, 2012c).
(d): Nose skin swabs have been reported to be more sensitive than boot swabs in poultry (P. Butaye, Veterinary and Agrochemical Research Centre, Belgium, personal communication, 2012).
(e): In certain MSs, the calf population to be monitored for MRSA may also comprise fattening veal calves older than 1 year

Relevant agent species to be reported

Strains of *Staphylococcus aureus* (*S. aureus*) resistant to virtually all available beta-lactam antimicrobials including methicillin (methicillin-resistant *S. aureus* (MRSA)) are to be reported. Information on the MRSA *spa*-types may also be reported if available, as well as further characterisation information related to clonal complexes and Multi-Locus Sequence Typing (MLST) types.

Type of specimen taken

Typically swabs from the lesions, biopsy, blood, dust, nasal swabs, milk samples.

Case definition/definition of a positive sample

***Staphylococcus* positive animal/sample/herd/flock/batch -** an animal/sample/herd/flock from which *Staphylococcus* has been isolated.
MRSA positive animal/sample/herd/flock/batch - an animal/sample/herd/flock from which MRSA has been isolated.

Diagnostic/analytical methods typically used

Currently, there is no internationally recognised standard method for detection of MRSA in animals.
Details should be provided in the MRSA text form on the diagnostic method used, including how verification of MRSA is carried out, in particular whether MRSA was detected by resistance testing of isolated *S. aureus* or by the use of selective media for MRSA.

Reporting the results in the tables

For reporting of data, use the table named '*Staphylococcus* in animals'.

Specific guidelines for reporting of data in the prevalence table:

- **Animal species** - for the specification of the animal species, the name of the animal species is first provided, then a more detailed breakdown information is given, such as the type of animals (wild, farmed, pet), production category (breeding, fattening animals), production period (during rearing period, adult), production system and housing conditions (not raised under controlled housing conditions, raised under controlled housing conditions), age (piglets, gilts, sows). For example: 'Cattle (bovine animals), meat production animals, calves (under 1 year*)'*. Generally, it is recommended to report information about the farmed or wild status of animal species in cases where the animal species can occur in both status.
- **Sampling stage** - to allow for comparability, data on the place or stage of sampling is reported by using a classification system provided in the pick list. The categories provide a list of main 'Places' or 'Stages' where samples may be taken e.g. at farm, at slaughterhouse, at retail.
- **Sampling context** - the information on the context of sampling (e.g. monitoring, surveillance) is reported by using a classification system in the pick list. A list of sampling programmes (e.g. monitoring) and a list of options for reporting the type of monitoring or survey (e.g. EFSA specifications, active or passive under the option monitoring) are provided.
- **Sampling details** - free text field that can be used to give further information on the sampling stage or context or other information in brief which is not covered by the columns present in the table.
- **Source of information** - the Institute (or laboratory) that has provided the data. Abbreviations should be clarified in the comments section or in the footnote unless already described in the 'Institute and laboratory List' under 'Edit report details'.
- **Sampling strategy** - the type of sampling should be reported in this column (e.g. 'objective sampling', 'suspect sampling').
- **Sampler** - who performed the sampling (e.g. competent authority ('official sampling') or industry ('HACCP and own checks')).
- **Sample type** - characterization of the sample category (i.e. 'animal sample', 'food sample', 'feed sample' or 'environmental sample') and the sample type (e.g. 'faeces', 'lymph nodes').
- **Sample origin** - this information allows for further characterization of the sample's origin (i.e. 'domestic', 'imported from outside EU', 'intra-EU trade').
- **Sampling unit** - use 'Herd', 'Flock', 'Holding', 'Slaughter batch' or 'Animal';
- **Units tested** - the number of sampling units that are analysed in total, and for which results are available;
- **Total units positive for *Staphylococcus*** - in this column, the total number of sampling units considered infected or colonised with *Staphylococcus*, based on the analyses results, should be inserted.
- **Total units positive for *S. aureus*, methicillin-resistant (MRSA)** - in this column, the total number of sampling units considered infected or colonised with MRSA, based on the analyses results, should be inserted.
- ***Spa*-type ….** - in this column the number of animals found positive for the specific MRSA *spa*-type should be reported. The information can be further refined indicating the number of animals found positive for a specific MRSA *spa*-type, clonal complex and/or MLST type. The

way the clonal complex and MLST type has been determined (whether from a performed experiment or inferred from other tests/online databases) may be clarified as a footnote.

- **MRSA, unspecified** - this column is used to indicate the number of sampling units for which the *spa*-type is unknown.

Sampling definitions are presented in Appendix B.

3.1.2. Methicillin-resistant *Staphylococcus aureus* in food

Table 2: Recommendations on the food categories and samples to collect for the MRSA monitoring

Food	MRSA
	Where to collect
Monitoring recommended to be performed on a voluntary basis (every third year)	
Fresh broiler meat	Cutting plant or at retail
Fresh turkey meat	Cutting plant or at retail
Fresh pork	Cutting plant or at retail
Fresh beef	Cutting plant or at retail
Fresh veal	Cutting plant or at retail
Raw milk and/or raw milk products	Dairy/processing plant or at retail

Relevant agent species to be reported

Strains of *S. aureus* resistant to virtually all available beta-lactam antimicrobials including methicillin-resistant *S. aureus* (MRSA) should be reported.
Information on the MRSA *spa*-types may also be reported if available, as well as further characterisation information related to clonal complexes and MLST types.

Case definition/definition of a positive sample

***Staphylococcus* positive sample/batch** - a sample/batch from which *Staphylococcus* has been isolated.

MRSA positive sample/batch - a sample/batch from which MRSA has been isolated.

Diagnostic/analytical methods typically used

Currently, there is no internationally recognised standard method for detection of MRSA in food. Details should be provided in the MRSA text form on the diagnostic method used, including how verification of MRSA is carried out, in particular whether MRSA was detected by resistance testing of isolated *S. aureus* or by the use of selective media for MRSA.

Reporting the results in the tables

For reporting of data, use the table named '*Staphylococcus* in food'.

Specific guidelines for reporting of data in the prevalence table:

- **Food category** - for the specification of the food, a high level categorization of foodstuffs should be first provided, thereafter reporting of more detailed information is allowed. For example: 'Milk, cows', raw milk for manufacture, intended for manufacturing of raw or low heat-treated products'. Where specific information is unavailable, one may use the unspecified option e.g. 'Milk from other animal species or unspecified'. This 'Unspecified' option should only be used when there is a specific need and no other option is available.

- **Sampling stage** - to allow for comparability, data on the place or stage of sampling should be reported by using a classification system provided in the pick list. The categories provide a list of main 'Places' or 'Stages' where samples may be taken e.g. at farm, at slaughterhouse or at retail.
- **Sampling context** - the information on the context of sampling (e.g. monitoring, surveillance) should be reported by using a classification system in the pick list. A list of sampling programmes (e.g. monitoring) and a list of options for reporting on the type of monitoring or survey (e.g. EFSA specifications, active or passive under the option monitoring) are provided.
- **Sampling details** - free text field that can be used to give further information on the sampling stage or context or other further information in brief which is not covered by the columns present in the table.
- **Source of information** - the Institute (or laboratory) that has provided the data. Abbreviations should be clarified in the comments section or in the footnote unless already described in the 'Institute and laboratory List' under 'Edit report details'.
- **Sampling strategy** - the type of sampling (i.e. 'census', 'convenient', 'objective', 'selective', 'suspect', or 'unspecified' sampling).
- **Sampler** - who performed the sampling should be reported here (e.g. 'official sampling' or 'HACCP and own checks').
- **Sample type** - characterization of the sample category (e.g. 'food sample') and the sample type (e.g. 'meat').
- **Sample origin** - this information allows for further characterization of the sample's origin (e.g. 'domestic', 'imported from outside EU', 'intra-EU trade').
- **Sampling unit** - for food 'Single' or 'Batch' should be used as the terms to be reported.
- **Sample weight** - the weight (in grams or millilitres) of the specimen used for analysis in the laboratory, e.g. 25 g. The sample weight should be reported as a number + space + unit of measure. Appropriate units of measure are g, mL and cm^2. No multiple weights ought to be reported on the same row. If results for specific weights are not known, the sample weight should be set to unknown.
- **Units tested** - the number of sampling units that are analysed in total, and for which results are available.
- **Total units positive for *Staphylococcus*** - in this column, the total number of sampling units considered contaminated with *Staphylococcus*, based on the analyses results, should be inserted.
- **Total units positive for *S. aureus*, methicillin resistant (MRSA)** - in this column, the total number of sampling units considered contaminated with MRSA, based on the analyses results, should be inserted.
- ***Spa*-type** - in this column the number of samples found positive for the specific *spa*-type should be reported. The information can be further refined indicating the number of animals found positive for a specific MRSA *spa*-type, clonal complex and/or multi-locus sequence typing (MLST) type. The way the clonal complex and MLST type has been determined (whether from a performed experiment or inferred from other tests/online databases) may be clarified as a footnote.
- ***S. aureus*, methicillin resistant (MRSA), unspecified** - this column is used to indicate the number of sampling units for which the *spa*-type is unknown.

3.2. Reporting on antimicrobial resistance

Detailed recommendations on the reporting on AMR have been issued by EFSA in the 'Technical specifications for the analysis and reporting of data an antimicrobial resistance in the European Union Summary Report' (EFSA, 2012a), 'Technical specifications on the harmonised monitoring and reporting of antimicrobial resistance in *Salmonella*, *Campylobacter* and indicator *Escherichia coli* and *Enterococcus* spp. bacteria transmitted through food' (EFSA, 2012b) and 'Technical specifications on the harmonised monitoring and reporting of antimicrobial resistance in methicillin-resistant *Staphylococcus aureus* in food-producing animals and food' (EFSA, 2012c). The present manual has been drafted taking these recommendations into account. Adherence to the guidance provided in this manual is strongly encouraged when reporting 2013 data.

When reporting data on antimicrobial resistance from animal species it is advisable to differentiate between different production sectors and production stages, which may differ substantially in terms of occurrence of antimicrobial resistance owing to important variety in management practices. It is acknowledged that submission of isolate-based data facilitates better reporting of resistance data by bacterial subtype (e.g. serovar for *Salmonella*) level and by production type/food category of origin.

3.2.1. Antimicrobial resistance monitoring in *Salmonella* spp.

Relevant animal species/food categories to be reported

- **Domestic fowl (*Gallus gallus*)**: it is advisable to report separately resistance data from breeding flocks, laying hen flocks and broiler flocks. When data are available, information on breeders of egg production line, breeders meat production line should? be reported separately.
- **Turkeys**: it is advisable to report separately resistance data from breeding flocks and fattening turkey flocks.
- **Pigs**: it is advisable to report separately resistance data from breeding pigs and growing/fattening pigs of the production stage. In addition, among growing pigs, the distinction between piglets, weaners to growers and fattening pigs is also recommended where information is available.
- **Cattle**: it is advisable to report separately resistance data from calves (under 1 year), young bovines (between 1 year and 24 months) and adult cattle (>24 months). The distinctions between veal calves/other calves, young bovines of dairy and meat sectors, dairy cattle and beef cattle are also encouraged where data are available. Those MSs monitoring AMR in fattening veal calve populations, that are older than 12 months of age and typically raised for the production of *rosé* veal, may report data under the new category veal calves (at or above 1 year).
- **Meat**: it is advisable to report resistance data from broiler meat, turkey meat, pig meat and bovine meat.
- **Other food-producing animal species** (e.g. geese, duck, sheep and goats) **and meat thereof**: it is advisable to report resistance data where data are available.

Relevant *Salmonella* serovars to be reported

It is recommended to report detailed resistance data on *Salmonella*, distinguishing between a number of serovars chosen because of public health relevance and/or epidemiological interest in the animal populations considered. For validation purposes, the category '**Other serovars**' should be used to include all the isolates tested that do not belong to the serovars individually reported.

In the quantitative antimicrobial susceptibility tables, the following *Salmonella* serovars of public health importance should be reported.

Table 3: *Salmonella* serovars of public health importance to be reported

Animal species/Animal population/Food categories	*Salmonella* serovar (Level 2 term)
All animal species and Derived meat	*S.* Typhimurium
	Monophasic *S.* Typhimurium
Gallus gallus (fowl): Breeders of egg production line, breeders of broiler production line, laying hens, broilers and Broiler meat	*S.* Enteritidis
	S. Agona
	S. Virchow
	S. Hadar
	S. Kentucky
	S. Infantis
Broilers and Broiler meat	*S.* Java[(a)]
Turkeys and Turkey meat	*S.* Enteritidis
	S. Agona
	S. Kentucky
	S. Newport
	S. Saintpaul
Pigs: breeding pigs, piglets, weaners to growers, fattening pigs,and Pig meat	*S.* Derby
	S. Agona
Cattle: dairy cattle, beef cattle and Bovine meat (veal or beef)	*S.* Dublin
	S. Infantis

(a): Formerly called *S.* Paratyphi B var. Java

The other *Salmonella* serovars should be grouped together for all animal or food origins as 'Other serovars'.

Recommended antimicrobials to be reported

- Ampicillin
- Cefotaxime
- Chloramphenicol
- Ciprofloxacin
- Gentamicin
- Nalidixic acid
- Streptomycin
- Sulfonamides[9]
- Tetracyclines
- Trimethoprim[9]

The antimicrobial pick list has been extended to also include antimicrobial substances recently recommended for susceptibility testing (EFSA, 2012b).

MSs that intend to report beta-lactam resistant phenotypes (ESBL, AmpC and/or carbapenemase) for *Salmonella* spp. and *Salmonella* serovars, as recommended in the recent EFSA technical specifications (EFSA, 2012b), may send proposals for adding the specific terms to the pick list.

[9] Trimethoprim and sulfonamides should be reported separately

3.2.2. Antimicrobial resistance monitoring in *Campylobacter* spp.

Relevant animal species/animal populations/food categories to be reported

- **Domestic fowl (*Gallus gallus*)**: it is advisable to report separately data from breeding flocks, laying hen flocks and broiler flocks or broilers at slaughter.
- **Turkeys**: it is advisable to report separately data from breeding flocks and fattening turkey flocks or turkeys at slaughter.
- **Pigs**: it is advised to report separately data from breeding pigs and growing/fattening pigs;
- **Cattle**: it is advisable to report separately data from calves (under 1 year), young bovines (between 1 year and 24 months) and adult cattle (>24 months). The distinctions between veal calves/other calves, young bovines of dairy and meat sectors, dairy cattle and beef cattle are also encouraged where data are available. MSs monitoring AMR in fattening veal calve populations, that are older than 12 months of age and typically raised for the production of *rosé* veal, may report data under the new category veal calves (at or above 1 year).
- **Meat**: it is advisable to report resistance data from broiler meat, turkey meat, pig meat and bovine meat.

Relevant agent species to be reported

C. jejuni and *C. coli* separately. Reporting of susceptibility data for *Campylobacter* spp. overall is discouraged because resistance patterns vary for different species.

Recommended antimicrobials to be reported

For *C. jejuni* and *C. coli* it is recommended that results are reported for:

- Erythromycin
- Ciprofloxacin
- Tetracyclines
- Streptomycin
- Gentamicin

The antimicrobial pick list has been also extended to include antimicrobial substances recommended for complementary susceptibility testing (EFSA, 2012b, c).

3.2.3. Antimicrobial resistance monitoring in indicator commensal *E. coli* (non-pathogenic)

Relevant animal species/animal populations/food categories to be reported

- **Domestic fowl (*Gallus gallus*)**: it is advisable to report separately data from breeding flocks, laying hen flocks and broiler flocks or broilers at slaughter.
- **Turkeys**: it is advisable to report separately data from breeding flocks and fattening turkey flocks or turkeys at slaughter.
- **Pigs**: it is advisable to report separately data from breeding pigs and growing/fattening pigs.
- **Cattle**: it is advisable to report separately data from calves (under 1 year), young bovines (between 1 year and 24 months) and adult cattle (>24 months). The distinctions between veal calves/other calves, young bovines of dairy and meat sectors, dairy cattle and beef cattle are also encouraged where data are available. MSs monitoring AMR in fattening veal calve populations, that are older than 12 months of age and typically raised for the production of *rosé* veal, may report data under the new category veal calves (at or above 1 year).
- **Meat**: it is advisable to report resistance data from broiler meat, turkey meat, pig meat and bovine meat.

Recommended antimicrobials to be reported

- Ampicillin
- Cefotaxime
- Chloramphenicol
- Ciprofloxacin
- Gentamicin
- Nalidixic acid
- Streptomycin;
- Sulfonamides[10]
- Tetracyclines
- Trimethoprim[10]

The antimicrobial pick list has been extended to include antimicrobial substances recently recommended for susceptibility testing (EFSA, 2012b; EFSA, 2012c)

3.2.3.1. Specific monitoring of ESBL- or AmpC- or carbapenemase producing *E. coli*

MSs that intend to report beta-lactam resistant phenotypes (ESBL, AmpC and/or carbapenemase) for *E. coli*, as recommended in the EFSA technical specifications (EFSA, 2012b), may send proposals for adding the specific terms to the pick list.

For detection of ESBL- or AmpC-producing *E. coli* the method shall start by a pre-enrichment step, followed by inoculation on McConkey agar containing a third generation cephalosporin in a selective concentration according to the most recent version of the detailed protocol for standardization of the European Union Reference Laboratory for Antimicrobial Resistance[11]. The microbial species *E. coli* shall be identified using an appropriate method.

A MS may decide, based on the epidemiological circumstances, to test in parallel an additional selective plate that inhibits for the growth of AmpC producing *E. coli* to facilitate the specific detection of ESBLs producing *E. coli*. When using this possibility, the results of the additional selective plate that inhibits for growth of AmpC producing *E. coli* shall be kept separately when reported.

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

One presumptive ESBL- or AmpC- or carbapenemase-producing *E. coli* obtained from each positive caecal sample and meat sample shall be tested on the first panel of antimicrobials in accordance with Table 4 and further submitted to extended susceptibility testing if they are resistant to cefotaxime, ceftazidime or meropenem based on the interpretative criteria listed in Table 4.

[10] Trimethoprim and sulfonamides should be reported separately.
[11] www.crl-ar.eu

3.2.4. AMR monitoring in indicator commensal *Enterococcus* spp. (non-pathogenic)

Relevant animal species/food categories to be reported

- **Domestic fowl (*Gallus gallus*)**: it is advisable to report separately data from breeding flocks, laying hen flocks and broiler flocks or broilers at slaughter.
- **Turkeys**: it is advisable to report separately data from breeding flocks and fattening turkey flocks or turkeys at slaughter.
- **Pigs**: it is advisable to report separately data from breeding pigs and growing/fattening pigs.
- **Cattle**: it is advisable to report separately data from calves (under 1 year), young bovines (between 1 year and 24 months) and adult cattle (>24 months). The distinctions between veal calves/other calves, young bovines of dairy and meat sectors, dairy cattle and beef cattle are also encouraged where data are available. Those MSs monitoring AMR in fattening veal calve populations, that are older than 12 months of age and typically raised for the production of *rosé* veal, may report data under the new category veal calves (at or above 1 year).
- **Meat**: it is advisable to report resistance data from broiler meat, turkey meat, pig meat and bovine meat.

Relevant agent species to be reported

Enterococcus faecium (*E. faecium*) and *Enterococcus faecalis* (*E. faecalis*) separately.

Recommended antimicrobials to be reported

Ampicillin
Chloramphenicol
Erythromycin
Gentamicin
Linezolid;
Quinopristin/dalfopristin
Streptomycin
Tetracyclines
Vancomycin

The antimicrobial pick list has been extended to include antimicrobial substances recommended for susceptibility testing (EFSA, 2012b).

3.2.5. Antimicrobial resistance monitoring in MRSA

Relevant animal species/food categories to be reported

Monitoring recommended to be performed consistently on a regular basis (every third year):

- **Broilers** at farm, boot swabs.
- **Fattening pigs**: slaughterhouse/**farm, pool of** nostril swabs/boot swab.
- **Turkeys**: it is advisable to report separately data from breeding flocks and fattening turkey flocks or turkeys at slaughter.
- **Pigs**: it is advisable to report separately data from breeding pigs and growing/fattening pigs.
- **Cattle: it is advisable to report separately data from calves (under 1 year), young bovines** (between 1 year and 24 months) and adult cattle (>24 months). The distinctions between veal calves/other calves, young bovines of dairy and meat sectors, dairy cattle and beef cattle are also encouraged where data are available. MSs monitoring AMR in fattening veal calve populations, that are older than 12 months of age and typically raised for the production of *rosé* veal, may report data under the new category veal calves (at or above 1 year).

- **Meat**: it is advisable to report resistance data from broiler meat, turkey meat, pig meat and bovine meat.

Recommended antimicrobials to be reported

The proposed lists of antimicrobials to be included in AMR monitoring in MRSA (EFSA, 2012c) are the following:

Recommended set:

Cefoxitin
Chloramphenicol
Ciprofloxacin
Clindamycin
Erythromycin
Gentamicin
Linezolid
Mupirocin
Quinupristin/Dalfopristin
Sulfamethoxazole/Trimethoprim
Tetracycline
Tiamulin
Vancomycin

Optional set:

Ceftobiprole
Kanamycin
Tigecycline
Fusidic acid
Daptomycin

4. Diagnostic/analytical methods typically used

Different types of methods are used in antimicrobial resistance testing for *Salmonella* and indicator bacteria: disk diffusion, agar dilution, broth dilution and E-test®. For *Campylobacter*, only dilution methods are considered reproducible.

Standard methods for antimicrobial susceptibility testing are given by the Clinical and Laboratory Standards Institute (CLSI) (CLSI standard M31 - A3 (CSLI, 2008)) and the European Committee on Antimicrobial Susceptibility Testing (EUCAST).

For *Salmonella* the dilution method should be used according to the methods described by the CLSI, accepted as international reference method (ISO standard 20776 - 1:2006 (ISO, 2006)), as stated in Commission Decision 2007/407/EC[12].

For *Campylobacter* dilution methods should be used according to the NCCLS M45 - A (CLSI, 2006), M100 - S17 (CLSI, 2007), or the methods described in the CLSI guidelines M31 - A3 (CSLI, 2008).

For indicator bacteria (*E. coli* and *Enterococci*) the international reference standard ISO 20776 - 1:2006 (ISO, 2006) shall be used.

The cut-off values should be reported.

In the present manual the term 'cut-off value' is consistent with the EFSA reports on the technical specifications for harmonised monitoring and reporting as well as with Commission Implementing Decision 2013/652/EU[13], which recommends to use the epidemiologic cut-off values for monitoring.

Member States shall test the antimicrobials and interpret the results using the epidemiological cut-off values and the concentration range that shown in Tables 4, 5 and 6, to determine the susceptibility of *Salmonella* spp, *C. coli*, *C. jejuni*, indicator commensal *E. coli*, *E. faecalis* and *E. faecium.*

[12] Commission Decision 2007/407/EC of 12 June 2007 on a harmonised monitoring of antimicrobial resistance in *Salmonella* in poultry and pigs. OJ L 153, 14.6.2007, p. 26–29.

[13] 2013/652/EU: Commission Implementing Decision of 12 November 2013 on the monitoring and reporting of antimicrobial resistance in zoonotic and commensal bacteria. OJ L 303, 14/11/2013, p. 26–39.

Table 4: Panel of antimicrobial substances to be included in AMR monitoring, EUCAST interpretative thresholds for resistance and concentration ranges to be tested in *Salmonella* spp. and indicator commensal *E. coli (*First panel)

Antimicrobial	*Salmonella* Interpretative thresholds of AMR		*E. coli* Interpretative thresholds of AMR		Range of concentrations (mg/L) (No. of wells in brackets)
	ECOFF[(a)]	Clinical breakpoint[(b)]	ECOFF[(a)]	Clinical breakpoint[(b)]	
Ampicillin	> 8	> 8	> 8	> 8	1-64 (7)
Cefotaxime	> 0.5	> 2	> 0.25	> 2	0.25-4 (5)
Ceftazidime	> 2	> 4	> 0.5	> 4	0.5-8 (5)
Nalidixic acid	> 16	NA	> 16	NA	4-128 (6)
Ciprofloxacin	> 0.064	> 1	> 0.064	> 1	0.015-8 (10)
Tetracycline	> 8	NA	> 8	NA	2-64 (6)
Gentamicin	> 2	> 4	> 2	> 4	0.5-32 (7)
Trimethoprim	> 2	>4	> 2	> 4	0.25-32 (8)
Sulfamethoxazole	NA	NA	> 64	NA	8-1 024 (8)
Chloramphenicol	> 16	> 8	> 16	> 8	8-128 (5)

NA: not available
(a): EUCAST epidemiological cut-off values
(b): EUCAST clinical resistance breakpoints

Table 5: Panel of antimicrobial substances to be included in AMR monitoring, EUCAST interpretative thresholds for resistance and concentration ranges to be tested in *C. jejuni and C. coli*

Antimicrobial	*C. jejuni* Interpretative thresholds of AMR		*C. coli* Interpretative thresholds of AMR		Range of concentrations (mg/L) (No. of wells in brackets)
	ECOFF[(a)]	Clinical breakpoint[(b)]	ECOFF[(a)]	Clinical breakpoint[(b)]	
Erythromycin	> 4	>4	> 8	> 8	1-128 (8)
Ciprofloxacin	> 0.5	> 0.5	> 0.5	> 0.5	0.12-16 (8)
Tetracycline	> 1	> 2	> 2	> 2	0.5-64 (8)
Gentamicin	> 2	NA	> 2	NA	0.12-16 (8)
Nalidixic acid	> 16	NA	> 16	NA	1-64 (7)
Streptomycin	> 4	NA	> 4	NA	0.25-16 (7)

NA: not available
(a): EUCAST epidemiological cut-off values
(b): EUCAST clinical resistance breakpoints

Table 6: Panel of antimicrobial substances to be included in AMR monitoring, EUCAST interpretative thresholds for resistance and concentration ranges to be tested in *E. faecalis* and *E. faecium*

Antimicrobial	*E faecalis* Interpretative thresholds of AMR		*E. faecium* Interpretative thresholds of AMR		Range of concentrations (mg/L) (No. of wells in brackets)
	ECOFF[(a)]	Clinical breakpoint[(b)]	ECOFF[(a)]	Clinical breakpoint[(b)]	
Gentamicin	> 32	NA	> 32	NA	8-1 024 (8)
Chloramphenicol	> 32	NA	> 32	NA	4-128 (6)
Ampicillin	> 4	> 8	> 4	> 8	0.5-64 (8)
Vancomycin	> 4	> 4	> 4	> 4	1-128 (8)
Erythromycin	> 4	NA	> 4	NA	1-128 (8)
Quinupristin/ Dalfopristin	NA	NA	> 1	> 4	0.5-64 (8)
Tetracycline	> 4	NA	> 4	NA	1-128 (8)
Linezolid	> 4	> 4	> 4	> 4	0.5-64 (8)
Ciprofloxacin	> 4	NA	> 4	NA	0.12-16 (8)

NA: not available
(a): EUCAST epidemiological cut-off values
(b): EUCAST clinical resistance breakpoints

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Appendix A. GENERAL DEFINITION

Antimicrobial – A drug which, at low concentrations, exerts an action against microbial pathogens and exhibits selective toxicity towards them[14]. Antimicrobials typically include antibiotics but also antivirals and other drugs effective against microorganisms.

Antibiotic - substance produced by or derived from a microorganism, which destroys or inhibits the growth of other microorganisms.

Antimicrobial resistance - the ability of microorganisms of certain species to survive or even grow in the presence of a given concentration of an antimicrobial agent that is usually sufficient to inhibit or kill microorganisms of the same species (Dir. 2003/99/EC). Resistance against an antimicrobial is considered to be present if the Minimum Inhibitory Concentration (MIC) exceeds the breakpoint or the epidemiological cut-off value.

Cut-off value - threshold value selected for distinguishing between negative and positive results, may include indeterminate or suspicious zone.

Microorganisms - bacteria, viruses, yeasts, moulds, algae, parasitic protozoa, microscopic parasitic helminths, and their toxins and metabolites (Reg. (EC) No 2073/2005).

Source of information - the institute (or laboratory or other organisation) that has provided the data.

Zoonoses - any disease and/or infection which is naturally transmissible directly or indirectly between animals and humans (Dir. 2003/99/EC).

[14] Opinion of the Scientific Steering Committee on Antimicrobial Resistance 28 May 1999. Available online: http://ec.europa.eu/food/fs/sc/ssc/out50_en.pdf

Appendix B. SAMPLING DEFINITIONS

Batch - group or set of identifiable products obtained from a given process under practically identical circumstances and produced in a given place within one defined production period (Reg. (EC) No 853/2004[15]).

Sample - set composed of one or several units or a portion of matter selected by different means in a population or in an important quantity of matter, which is intended to provide information on a given characteristic of the studied population or matter and to provide a basis for a decision concerning the population or matter in question or concerning the process which has produced it (Reg. (EC) No 2073/2005).

Sample origin - information on where the sample originated from (i.e. domestic, imported from outside EU, intra EU trade).

Sample type - represents the characterization of the sample category (e.g. animal, food, feed or environmental sample) and the sample type (e.g. faeces, lymph nodes).

Sample weight - the weight (in grams or millilitres or cm^2) **of the specimen used for analysis in the laboratory**. The sample weight should be reported as a number + space + unit of measure. Appropriate units of measure are g, mL and cm^2. No multiple weights ought to be reported on the same row. If results for specific weights are not known, the sample weight should be set to unknown.

Sampling frame - complete list of all units of the population, which can be sampled.

Sampling strategy - planned procedure for selecting samples from a population and for conducting the sampling in order to obtain the information needed.

Sampling unit - the unit which the specimens taken represent and which is considered either infected (contaminated) or not, based on the analyses result. For animal data, the sampling unit may be 'Animal', 'Flock', 'Herd', 'Holding' or 'Slaughter batch'; for food and feed data, the sampling unit might be 'Single' or 'Batch'.

Single - means a foodstuff or a feedingstuff comprised of one unit or a portion of matter e.g. a package, a carcase, a piece of cheese. It does not represent the entire batch (of production or consignment).

Specimen - unit or portion of a matter which is sampled and intended to be analysed.

[15] Regulation (EC) No 853/2004 of the European Parliament and of the Council of 29 April 2004 laying down specific hygiene rules for food of animal origin. OJ L 139, 30.4.2004, p. 55–205.

Appendix C. DEFINITIONS REGARDING THE SAMPLING CONTEXT

Control programme - programme applying measures designed to reduce the frequency of existing infection or contamination to levels biologically and/or economically justifiable or otherwise of little consequence.

Eradication programme - programme applying measures aimed at eliminating selected zoonotic agents from a defined area. In the context of Directive 77/391/EEC[16], the eradication programmes are so devised that, on their completion, herds are classified as brucellosis/tuberculosis officially free.

HACCP (Hazard Analysis and Critical Control Point) - programme designed to effectively control processes by identifying Critical Control Points (CCP), establishing critical limits for each CCP, monitoring CCP, gathering data, keeping records, implementing corrective actions and verification procedures. HACCP is applied by the food or feed business operators (Codex Alimentarius).

Monitoring - system of collecting, analysing and disseminating data on the occurrence of zoonoses, zoonotic agents and antimicrobial resistance related thereto. As opposed to surveillance, no active control measures are taken when positive cases are detected (Dir. 2003/99/EC).

Monitoring - active - active monitoring programme of zoonotic agents or antimicrobial resistance in food and animals is based on random sampling strategies of the population of interest, stratified according to the relevant subcategories of the population. The sampling strategy should ensure the sample representativeness of the population of interest, and the robustness of the sampling method.

Monitoring - passive - passive monitoring programme of zoonotic agents or antimicrobial resistance includes information from diagnostic testing, or a representative selection of this information. Data on prevalence of the zoonotic agents and on antimicrobial resistance provided by passive monitoring programme typically derive from diseased animals.

Monitoring - EFSA specifications - a monitoring system following harmonised technical specifications prepared by EFSA.

Official control - any form of control that the competent authority or the Community performs for the verification of compliance with feed and food law, animal health and animal welfare rules (Reg. (EC) No 882/2004[17]).

Official sampling - sampling performed under control of the competent authority.

Objective sampling - planned strategy based on the selection of a random sample, which is statistically representative of the population to be analysed. Each unit, within the framework population, has a specified probability of being selected. This strategy provides with data from which statistical inference can be implemented. That means that the results inferred are comparable.Objective sampling is often the case in monitoring and surveillance schemes as well as surveys.

Sampler - who performs the sampling (e.g. competent authority ('official sampling') or industry ('HACCP or own checks')).

Selective sampling - planned strategy where the selection of the sample is from previously defined 'high-risk' population groups. Samples are normally selected to either illustrate or document

[16] Council Directive 77/391/EEC of 17 May 1977 introducing Community measures for the eradication of brucellosis, tuberculosis and leucosis in cattle. OJ L 145, 13.6.1977, p. 44–47.

[17] 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. 1–141.

unsatisfactory conditions or suspected adulteration of a product. The sampling is deliberately biased and is directed at the particular products or manufacturers. The sampling procedure can be random or not. The specification of the 'high-risk' population comes from either scientific studies or previous analysis and information of other regions or countries. The comparability of the results lies on both the definition of the population to be analysed and the way the samples have been drawn.

Suspect sampling - unplanned selection of a sample, where the individual units are selected based on the recent judgement and experience regarding the population, lot, or sampling frame, e.g. earlier positive samples. The samples obtained from this procedure are not randomly extracted.

Census - strategy where all units of the population are sampled.

Convenience sampling - is used in exploratory research where the researcher is interested in getting an inexpensive approximation of the truth. The samples are selected because they are convenient. This non probability method is often used during preliminary research efforts to get a gross estimate of the results, without incurring the cost or time required to select a random sample. This methodology is potentially subject to serious bias.

Sampling strategy - planned procedure for selecting samples from a population and for conducting the sampling in order to obtain the information needed.

Surveillance - a careful observation of one or more food or feed businesses, food or feed business operators or their activities (in the context of the food and feed control Reg. (EC) No 882/2004). In general, it means a close and continuous observation for the purpose of control. As opposed to monitoring, active control measures are taken when positive cases are detected. This type of programme does not necessarily have a defined target for diseases/contamination occurrence reduction.

Survey - study involving a sample of units selected from a larger, well-delineated population. This (target) population is the entire set of units to which findings of the survey are to be extrapolated. The units to examine are to be selected randomly (Rothman, 1986 and Noordhuizen et al., 2001).

Survey - EU baseline survey - a study involving a sample of units selected from a larger, well-delineated population. This (target) population is the entire set of units to which findings of the survey are to be extrapolated. The units to examine are to be selected randomly.

Appendix D. DEFINITIONS OF FOODSTUFFS

Carcase - the body of an animal after slaughter and dressing (Reg. (EC) No 853/2004).

Cutting plant - an establishment used for boning and/or cutting up meat (Reg. (EC) No 853/2004).

Dairy products - processed products resulting from the processing of raw milk or from the further processing of such processed products (Reg. (EC) No 853/2004).

Egg products - processed products resulting from the processing of eggs, or of various components or mixtures of eggs, or from the further processing of such processed products (Reg. (EC) No 853/2004).

Eggs - eggs in shell, other than broken, incubated or cooked eggs, that are produced by farmed birds and are fit for direct human consumption or for the preparation of egg products (Reg. (EC) No 853/2004).

Food (or foodstuff) - any substance or product, whether processed, partially processed or unprocessed, intended to be, or reasonably expected to be, ingested by humans (Reg. (EC) No 178/2002[18]).

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 (Reg. (EC) No 853/2004).

Meat - edible parts of the animals below mentioned, including blood (Reg. (EC) No 853/2004).

Meat preparations - fresh meat, including meat that has been reduced to fragments, which has had foodstuffs, seasonings or additives added to it or which has undergone processes insufficient to modify the internal muscle fibre structure of the meat and thus to eliminate the characteristics of fresh meat (Reg. (EC) No 853/2004).

Meat products - processed products resulting from the processing of meat or from the further processing of such processed products, so that the cut surface shows that the product no longer has the characteristics of fresh meat (Reg. (EC) No 853/2004).

Minced meat - boned meat that has been minced into fragments and contains less than 1 % salt (Reg. (EC) No 853/2004).

Offal - fresh meat other than that of the carcase, including viscera and blood (Reg. (EC) No 853/2004).

Packing centre - establishment where eggs are graded by quality and weight (Reg. (EC) No 853/2004).

Processed products - foodstuffs resulting from the processing of unprocessed products. These products may contain ingredients that are necessary for their manufacture or to give them specific characteristics (Reg. (EC) No 852/2004[19]).

[18] 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.

[19] Regulation (EC) No 852/2004 of the European Parliament and of the Council of 29 April 2004 on the hygiene of foodstuffs. OJ L 139, 30.4.2004, p. 1–54.

Processing - any action that substantially alters the initial product, including heating, smoking, curing, maturing, drying, marinating, extraction, extrusion or a combination of those processes (Reg. (EC) No 852/2004).

Products of animal origin - food of animal origin, including honey and blood; live bivalve molluscs, live echinoderms, live tunicates and live marine gastropods intended for human consumption; and other animals destined to be prepared with a view to being supplied live to the final consumer (Reg. (EC) No 853/2004).

Raw milk - milk produced by the secretion of the mammary gland of farmed animals that has not been heated to more than 40°C or undergone any treatment that has an equivalent effect (Reg. (EC) No 853/2004).

Ready-to-eat food - food intended by the producer or the manufacturer for direct human consumption without the need for cooking or other processing effective to eliminate or reduce to acceptable level microorganisms of concern (Reg. (EC) No 2073/2005).

Slaughterhouse - establishment used for slaughtering and dressing animals, the meat of which is intended for human consumption (Reg. (EC) No 853/2004).

Unprocessed products - foodstuffs that have not undergone processing, and includes products that have been divided, parted, severed, sliced, boned, minced, skinned, ground, cut, cleaned, trimmed, husked, milled, chilled, frozen, deep-frozen or thawed (Reg. (EC) No 852/2004).

Appendix E. DEFINITIONS OF ANIMALS

Animal - any animal of the species referred to in EU Directives (Dir. 64/432/EEC, Dir. 91/68/EEC and Dir. 92/102/EEC[20]).

Animals for slaughter - bovine animal (including the species *Bison bison* and *Bubalus bubalus*), swine or animals of the ovine or caprine species intended to be taken to a slaughterhouse or assembly centre from which it may only move to slaughter (Dir. 64/432/EEC and Dir. 91/68/EEC).

Animals for breeding or production - bovine animals (including the species *Bison bison* and *Bubalus bubalus*) and swine other than animals for slaughter, including those intended for breeding, milk or meat production, or draft purposes, shows or exhibition with the exception of animals taking part in cultural and sporting events (Dir. 64/432/EEC).

Breeding poultry - poultry 72 hours old or more, intended for the production of hatching eggs (Dir. 90/539/EEC[21]).

Calves - domestic animals of the bovine species not exceeding a live weight of 300 kg, which do not yet have their second teeth (Dec. 94/433/EC[22]).

Calves for slaughter - cattle less than 12 months old intended for slaughter as calves (Dec. 94/433/EC).

Cows - female bovine animals which have already calved (Dec. 94/433/EC).

Cows, dairy - cows which are kept exclusively or principally to produce milk for human consumption and/or for processing into dairy products. Includes cull dairy cows (whether or not they are fattened between their last lactation and slaughter) (Dec. 94/433/EC).

Day-old chicks - all poultry less than 72 hours old, not yet fed; however, Barbary ducks may be fed (Dir. 90/539/EEC).

Epidemiological unit - group of animals which is of epidemiological importance in terms of the transmission and maintenance of infection.

Ewes, Milk - ewes which are kept exclusively or principally to produce milk for human consumption and/or processing into dairy products. This includes cast milk sheep (whether fattened or not between their last lactation and slaughtering).

Ewes, Other - ewes other than milk ewes, to be included in production animals.

Ewes and ewe lambs put to the ram - females of the ovine species which have already lambed at least once as well as those which have been put to the ram for the first time.

[20] Council Directive 92/102/EEC of 27 November 1992 on the identification and registration of animals. OJ L 355, 5.12.1992, p. 32–36.

[21] Council Directive 90/539/EEC of 15 October 1990 on animal health conditions governing intra-Community trade in, and imports from third countries of, poultry and hatching eggs. OJ L 303, 31.10.1990, p. 6–28.

[22] Commission Decision 94/433/EC of 30 May 1994 laying down detailed rules for the application of Council Directive 93/24/EEC as regards the statistical surveys on cattle population and production, and amending the said Directive. OJ L 179, 13.7.1994, p. 27–32.

Flock - all poultry of the same health status kept on the same premises or in the same enclosure and constituting a single epidemiological unit; in the case of housed poultry, this includes all birds sharing the same airspace (Reg. (EC) No 2160/2003[23]).

Goats - domestic animals of the species *Capra*.

Heifers - female non-calve bovine animals which have not yet calved (based on Dec. 94/433/EC).

Heifers for slaughter - heifers bred for meat production (Dec. 94/433/EC).

Heifers for breeding purposes - heifers raised for breeding and intended to replace cows.

Herd - an animal or group of animals kept on a holding as an epidemiological unit (Reg. (EC) No 2160/2003); if more than one herd is kept on a holding, each of these herds shall form a distinct unit and shall have the same health status (Dir. 64/432/EEC).

Holding - any establishment, construction or, in the case of an open-air farm, any place in which animals are held, kept or handled (Dir. 92/102/EEC).

Lambs - male or female sheep under 12 months of age.

Meat production animals (bovines) - bovine animals, other than calves, kept exclusively for the production of meat and including cows, heifers and bulls.

Milk production holding - establishment where one or more farmed animals are kept to produce milk with a view to placing it on the market as food (Reg. (EC) No 853/2004).

Pigs - domestic animals of the species *Suis*.

Poultry - fowl, turkeys, guinea fowl, ducks, geese, quails, pigeons, pheasants and partridges reared or kept in captivity for breeding, the production of meat or eggs for consumption, or for restocking supplies of game (Dir. 90/539/EEC).

Sheep - domestic animals of the species *Ovis*.

Spent hens - hens that do adequately not perform their duty of breeding or egg laying.

Steers - male bovine animal castrated before sexual maturity.

[23] 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.

ABBREVIATIONS

AMR	Anti Microbial Resistance
CCP	Critical Control Points
CLSI	Clinical and Laboratory Standards Institute
CSV	Comma Separated Values
DCF	Data Collection Framework
EC	European Commission
ECDC	European Centre for Disease Prevention and Control
EEC	European Economic Community
EFSA	European Food Safety Authority
EMA	European Medicine Agency
EN	European Norm
EU	European Union
EUCAST	European Committee on Antimicrobial Susceptibility
HACCP	Hazard Analysis Critical Control Point
ISO	International Organization for Standardization
MIC	Minimum Inhibitory Concentration
MLST	Multi-Locus Sequence Typing
MRSA	Methicillin-resistant *Staphylococcus aureus*
MS	Member State of the European Union
NCCLS	National Committee for Clinical Laboratory Standards
spa	*Staphylococcus* protein A
XML	Extensible Markup Language