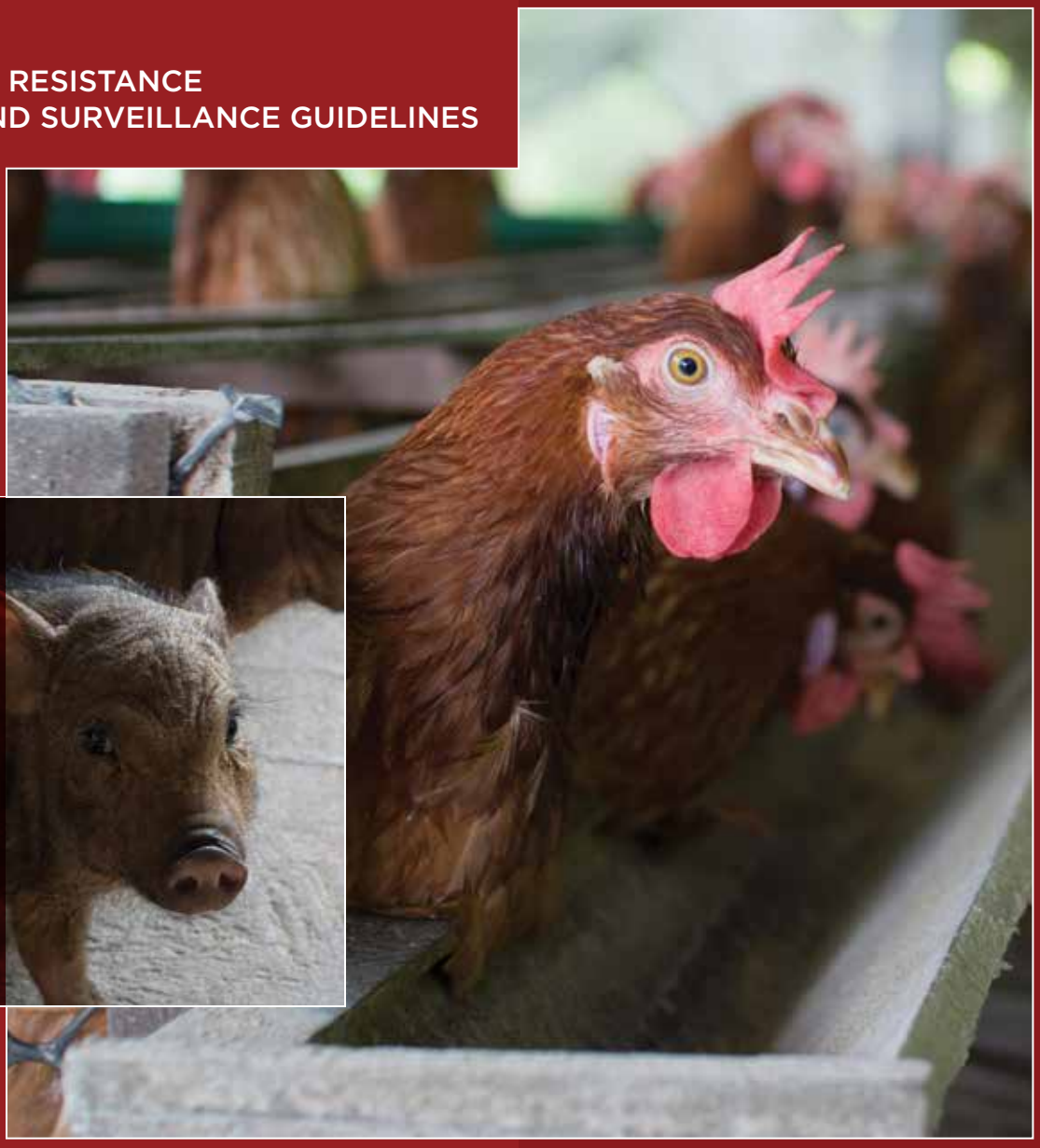
Food and Agriculture Organization of the United Nations

REGIONAL
ANTIMICROBIAL RESISTANCE
MONITORING AND SURVEILLANCE GUIDELINES



Volume 1

Monitoring and surveillance of antimicrobial resistance in bacteria from healthy food animals intended for consumption

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FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS
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PREPARATION OF THIS DOCUMENT

This publication is part of the series of regional guidelines for antimicrobial resistance (AMR) in agriculture. It is an output of the consultative process among global and regional stakeholders and experts, especially in Southeast Asia.

Part of that consultative process was the review of the initial draft guideline and further inputs from the Southeast Asia Antimicrobial Use and Antimicrobial Resistance Technical Advisory Group (SEA AMU/AMR TAG). Member countries (Cambodia, Lao People's Democratic Republic, Malaysia, Myanmar, Philippines, Singapore, Thailand, Viet Nam) as well as international experts and resource persons participated during the two-day review in Siem Reap Cambodia (20-21 November 2017). The international experts and resource persons included representatives from the Chulalongkorn University Veterinary Antimicrobial Resistance Cluster (CU VET AMR) in Thailand; the Japanese Veterinary Antimicrobial Resistance Monitoring System (JVARM) of the Japan Ministry of Agriculture, Forestry and Fisheries; New Zealand Institute of Environmental Science and Research (ESR); Utrecht University of The Netherlands; Ausvet of Australia; World Organisation for Animal Health (OIE); and the United States International Agency for Development (USAID). The resulting version of the guideline from this consultative review received additional comments and inputs from Angus Cameron, Jaap Wagenaar, Mayumi Kijima, Yueh Nuo Lim, Alessandro Partriarchi and Mariani Binti Hashim. Katharine Stark, an external peer reviewer, then reviewed the subsequent version of the guideline.

Another round of review was carried out in Bangkok, Thailand on 21-22 November 2018. Participants included member countries of the SEA AMU/AMR TAG, CU VET AMR, JVARM; ESR; Epidemia of Australia, the Clinical Laboratory Standards Institute (CLSI), National Monitoring Network for AMR in Animal Pathogens (RESAPATH) of the the French Agency for Food, Environmental and Occupational Health & Safety (ANSES). Partner organizations who also participated in the meeting included OIE, World Health Organization Southeast Asia Regional Office (WHO SEARO), USAID, Network of Aquaculture Centers of Asia (NACA), and FAO global and regional experts. Other participants included observers from SAFOSO of Switzerland and Mott MacDonald.

Finally, additional comments and inputs from the FAO Animal Production and Health Division in Rome, Italy were provided by Juan Lubroth, Alejandro Dorado-Garcia, Francesca Latronico, Nicolas Keck, Michael Trellis, Alice Green, Jeff Lejeune, and Suzanne Eckford.

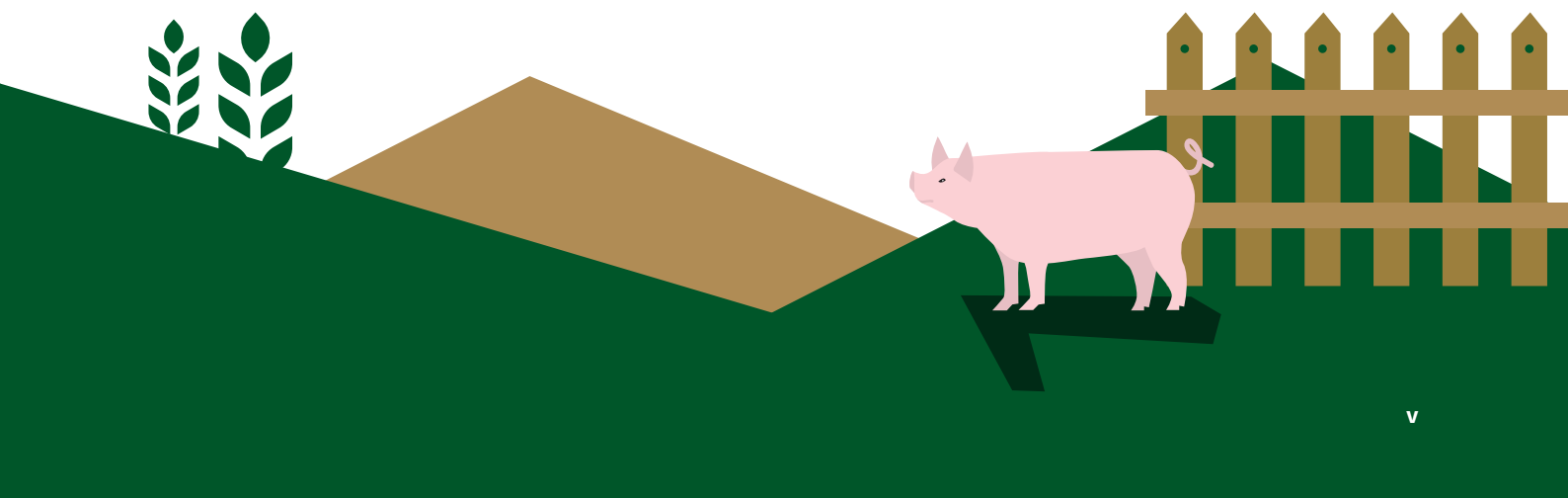
FAO RAP developed this guideline in collaboration with the CU VET AMR. The lead authors were Rungtip Chuanchuen, Taradon Luangtongkum, Saharuetai Jeamsripong, and Mary Joy Gordoncillo. Iljas Baker provided copy editing and proofreading services.

The United States Agency for International Development (USAID) funded the preparation and publishing of this guideline.

ABSTRACT

The animal health subsector within the agriculture sector is the gatekeeper of antimicrobial resistance (AMR) in livestock, aquaculture, animal products, and the immediate animal environment. In support of member countries taking responsibility for and moving forward with putting AMR monitoring and surveillance in place for the animal sector, the Food and Agriculture Organization of the United Nations Regional Office for Asia and the Pacific (FAO-RAP) developed a regional AMR surveillance framework, each pillar of which is complemented by a guideline to reinforce its progressive implementation. The first of this series, *Volume 1: Monitoring and surveillance of antimicrobial resistance in bacteria from healthy food animals intended for consumption,* is centered on healthy animals reaching consumers and on the protection of public health. It provides guidance on the design of AMR monitoring and surveillance, with particular emphasis on relevant epidemiology and laboratory methods, as well as AMR data management. It underscores the importance of the representativeness of samples to be obtained from apparently healthy animals and food products of animal origin so as to reflect an unbiased estimate of the prevalence of AMR in target organisms circulating in the major animal commodities of the countries of the region. The guideline encourages countries to initiate AMR surveillance regardless of their capacity, and it also provides guidance and recommended approaches to progressively move towards a regionally harmonized and standardized approach at the outset, which will be important for comparability and monitoring changes in the susceptibility of target bacteria to specific antimicrobials over the years to come. The guideline is also reinforced by auxiliary AMR surveillance planning tools and a regional template for AMR collection. This first guideline was prioritized in the development process because this interface represents the most salient pathway towards human exposure from the animal subsector. Other guidelines are also currently underway for AMR surveillance in bacterial pathogens from clinically ill animals, aquaculture and fisheries, the animal environment as well as antimicrobial use at the farm level.

CONTENTS



FOREWORD

Antimicrobial resistance (AMR) in bacterial organisms does not recognize biological, physical, or sectoral boundaries. Its potential transmission pathways are best addressed by disciplines that are often traditionally segregated. Collective and coordinated actions across these multiple disciplines can leverage the strengthened sectoral accountability for AMR mitigation. This ensures that the efforts of nations to address AMR will benefit from the expertise of each sector, with actions sustained by their respective mandates. For AMR monitoring and surveillance, the FAO Regional Office for Asia and the Pacific (FAO RAP) has embarked on facilitating the development of a series of regionally harmonized guidelines relevant to this sector.

This is the first of a series of publications on regional AMR monitoring and surveillance guidelines. **Regional Antimicrobial Resistance Monitoring and Surveillance Guideline Volume 1: Monitoring and surveillance of antimicrobial resistance in bacteria from healthy food animals intended for consumption** provides guidance on the design, planning, implementation, and data application relevant to monitoring AMR in bacteria from apparently healthy animals intended for human consumption. Although anchored in existing international standards, this guideline takes into account the unique settings of the region including the varying levels of advancement of member countries, their distinct animal population dynamics (e.g. predominance of smallholders, existence of live-bird markets and slaughter points, informal trade, live animal movements through porous borders), resources limitations, and other considerations specific to the region. This guideline can also serve as a tool for obtaining regionally harmonized information, bringing benefit to the individual efforts of member countries in the Asia-Pacific region. With objectives primarily centered on protecting public health, this guideline will help showcase the benefits and comparative advantage of a strengthened animal sector in addressing issues at the human-animal-environment interface, and ultimately its value in increasing the resilience of livelihoods to threats and crises.

We hope that this series of publications will further strengthen surveillance strategies in the region and ensure standardized and better AMR surveillance.

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FAO RAP also acknowledges with appreciation the member countries, international experts and resource persons, partners, external reviewers and colleagues from FAO headquarters and RAP for their contributions to this guideline.

In particular, FAO RAP acknowledges the participation and contribution of Cambodia, Lao People's Democratic Republic, Malaysia, Myanmar, Philippines, Singapore, Thailand, Viet Nam, CU VET AMR, JVARM, ESR, Ausvet, WHO SEARO, ESR, Utrecht University, RESAPATH, SAFOSO, Mott MacDonald, OIE, and USAID.

FAO RAP acknowledges inputs from Angus Cameron, Jaap Wagenaar, Mayumi Kijima, Yueh Nuo Lim, Alessandro Partriarchi, Mariani Binti Hashim, Juan Lubroth, Alejandro Dorado-Garcia, Francesca Latronico, Nicolas Keck, Michael Trellis, Alice Green, Jeff Lejeune, and Suzanne Eckford.

The completion of this publication was made possible through the support of and various contributions from FAO RAP staff: Katinka de Balogh, Lead Technical Officer for AMR; Wantanee Kalpravidh, Emergency Centre for Transboundary Animal Diseases (ECTAD) Asia Regional Manager; Carolyn Benigno, former AMR Regional Project Coordinator; Filip Claes, Regional Laboratory Coordinator; Aurelie Brioudes, Regional Surveillance Coordinator; Mary Joy Gordoncillo, Regional AMR Project Coordinator; Han Zifeng, AMU/AMR Project Officer; and Domingo Caro, Regional Advocacy and Development Communication Coordinator.

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ABBREVIATIONS AND ACRONYMS

AGISAR	WHO Advisory Group on Integrated Surveillance of Antimicrobial Resistance
AMR	Antimicrobial resistance
AMU	Antimicrobial usage
AST	Antimicrobial susceptibility testing
ATCC®	American Type Culture Collection
BHI	Brain heart infusion
CAMHB	Cation-adjusted Mueller–Hinton broth
CBP	Clinical breakpoint
CLSI	Clinical and Laboratory Standards Institute
DNA	Deoxyribonucleic acid
ECOFF	Epidemiological cut-off value
ECV	Epidemiological cut-off value
EFSA	European Food Safety Authority
ESBL	Extended Spectrum Beta-Lactamase
EUCAST	European Committee on Antimicrobial Susceptibility Testing
FAO	Food and Agriculture Organization of the United Nations
FAO RAP	FAO Regional Office for Asia and the Pacific
GAP	Global Action Plan on Antimicrobial Resistance
ISO	International Organization for Standardization
MALDI-TOF	Matrix-assisted laser desorption/ionization-time of flight
MHA	Mueller–Hinton agar
MIC	Minimum inhibitory concentration
MRD	Maximum recovery diluent
OIE	World Organisation for Animal Health
PCR	Polymerase chain reaction
QA	Quality assurance
QC	Quality control
SEA	Southeast Asia
WHO	World Health Organization

CHAPTER

1 INTRODUCTION TO THE GUIDELINE

1.1 The need for harmonized protocols for antimicrobial resistance testing and monitoring

Antimicrobial resistance (AMR) is a rising global concern that affects human, animal, and plant health, and food and the environment. Resistance to antimicrobial compounds commonly used in human and veterinary medicine has been increasing globally in both human and veterinary health settings. In fact, AMR in bacteria has become a major threat to public health globally.

Surveillance of AMR in bacteria from healthy animals intended for food consumption involves active monitoring of AMR in zoonotic and commensal bacteria in apparently healthy food-producing animals and in animal food products. Isolates obtained from routine food-borne surveillance programmes may also be used or appropriately stored for future use (i.e. a retrospective study) (EFSA, 2018). Data obtained from this type of surveillance will be important for understanding the epidemiology of AMR in the food chain and for monitoring the impact of antimicrobial usage in animals. It also provides essential data for risk analyses for both human and animal populations, and for the evaluation of interventions.

Currently, there are a number of AMR surveillance programmes involving the animal sector operational throughout the world. The lack of harmonization in sampling, susceptibility testing methods, antimicrobial agents tested, interpretive criteria and reporting often makes it difficult to compare data across programmes, which is an essential requirement in increasingly global

food chains. As AMR surveillance is being initiated progressively in many countries in Asia, it is important that efforts are made to ensure comparability of the resulting future data to maximize the potential value of the cumulative findings across the region. Member countries are thus encouraged to ensure that their respective initiatives follow a standardized and harmonized protocol for AMR monitoring and antimicrobial susceptibility testing (AST). The harmonized protocol for AMR testing and monitoring must provide robust science-based technical methodologies from sample collection through to data analysis and data reporting adapted to the regional setting. It should provide a quantitative analysis of temporal-based trends in the occurrence and spread of AMR and allow the identification of emerging or specific resistance patterns.

1.2 Monitoring and surveillance of antimicrobial resistance in bacteria from healthy food animals intended for consumption

This regional AMR surveillance guideline provides guidance for the development of an AMR surveillance plan for food-borne bacteria, underscoring the key elements for harmonized AMR data generation, data collation and reporting of findings, and taking into consideration the unique context of the region. It aims to provide guidelines for a harmonized scheme for AST and laboratory-based monitoring for AMR. The protocol was developed following a thorough review of the current guidance, recommendations, technical specifications and publications (EFSA, 2012b; EFSA, 2014; OIE, 2012; WHO, 2017) of the existing national AMR surveillance programmes (EFSA, 2008; USDA, 2014; NFI and SSI, 2016; Heffernan et al., 2011; NVAL, 2016), as it is intended to support the harmonization of a global AMR surveillance system.

Although these and other long-standing examples provided the technical framework for this guideline, the unique setting of the region and practical limitations were also taken into account. As AMR surveillance is a relatively new area of work for many of the government units involved in the implementation of their respective AMR national action plans, this comprehensive guideline was reinforced with concomitant initiatives to bridge gaps in knowledge and build essential capacities where AMR and AMR mitigation is concerned. This guideline also took into account the experiences and lessons of pilot AMR surveillance initiatives in Asia, with reviews by the FAO Regional Office for Asia and the Pacific (FAO RAP) and the members of the AMR Technical Advisory Group (AMR-TAG) of Southeast Asia.

It should be noted that this guideline only covers surveillance of AMR in bacteria from healthy terrestrial animals intended for consumption. Separate documents cover surveillance of AMR in animal bacterial pathogens (Guideline Volume 2), surveillance of AMR in aquatic animals (Guideline Volume 3), and surveillance of AMR in the animal enivronment (Guideline Volume 4), as well as documentation of antimicrobial usage (Guideline Volume 5).

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1.3 Purpose of monitoring and surveillance of antimicrobial resistance in bacteria from healthy animals

This document provides guidance for countries in Asia implementing monitoring and surveillance of AMR in bacteria associated with food animals for any, or all, of the purposes outlined in the OIE Terrestrial Animal Health Code Section 6.7.2 (OIE, 2012a). These are to:

- "assess and determine the trends and sources of antimicrobial resistance in bacteria;
- detect the emergence of new antimicrobial resistance mechanisms;
- provide the data necessary for conducting risk analyses as relevant to animal and human health;
- provide a basis for policy recommendations for animal and human health;
- provide information for evaluating antimicrobial prescribing practices and for prudent use recommendations [and provide feedback information to antimicrobial users];
- assess and determine the effects of actions to combat antimicrobial resistance".

Particularly focusing on monitoring and surveillance of AMR in microorganisms from healthy food animals and their products, this regional guideline document will further:

- assist countries to initiate establishing baseline data on the prevalence of resistance to antimicrobial agents in commensal bacteria and food-borne pathogens from food animals and their products;
- encourage cooperation among member countries; and
- guide the progressive work of the countries towards producing regionally harmonized AMR data.

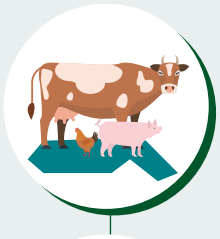
1.4 Scope of the guidance

This document provides detailed guidance on the harmonized antimicrobial susceptibility testing and monitoring of AMR in different bacterial species isolated from healthy food animal species and/or foods that originated from the animals. The guidance should be used for major food animal species including broilers, swine, cattle and their meat products (i.e. chicken meat, pork, beef). In this context, the selected food-borne pathogens Salmonella enterica and *Campylobacter* spp. (*C. jejuni* and *C. coli*) and the commensal bacteria *Escherichia coli* and *Enterococcus* spp. (E. faecium and E. faecalis) (See Section 2.4 and Table 2.1) are those considered most relevant to AMR monitoring from a public health perspective.

Figure 1.1 gives a brief overview of the decisions that will have to be made by those responsible for carrying out the surveillance activities. Further information on these decisions and activities is presented in the remainder of this guideline in the sections referred to in the figure.

Figure 1.1 Overview of decisions and activities in carrying out surveillance of AMR in bacteria from healthy food animals and their products.

WHAT ARE YOUR AMR SURVEILLANCE OBJECTIVES?



What is your target population? (Section 2.2)
Member countries should examine their livestock production systems on the basis of available information and assess which sources are likely to contribute most to a potential risk to animal and human health. Countries should prioritize the main food-producing animal species contributing to the most consumption yield in the country.

Where will you obtain your samples? (Section 2.3)
Countries should aim to conduct AMR monitoring and surveillance targeting animals at the end of their production cycle. This is normally most practical using abattoir sampling but may be done on-farm in some circumstances if it is simpler and cheaper. Surveillance of meat (domestic and/or imported) at retail outlets may be included as an additional option.

What is your sampling frame? (Section 2.5)
Where feasible, it is ideal to have at least 80 percent of the total target population (ranked highest to lowest) included in the sampling frame from which the actual samples will be drawn. When a suitable sampling frame is not available, proxy (or indirect) sampling may be used, such as list of villages or other geographic units deemed most appropriate.

How many samples do you need? (Section 2.6)
Various references are available and can be used for sample size calculation, however, it should be noted that this part of the planning should also benefit from the input of relevant units of the Competent Authority with this area of expertise (e.g. epidemiology section of the Veterinary Authority). Whatever approach is used, it should reflect the set objectives for the national AMR surveillance, and comply with the OIE Terrestrial Animal Health Code (2018) Chapter 6.8.4

What other considerations are there? (Section 2.7)
There are various considerations in the design of AMR surveillance and monitoring in food-borne bacteria from healthy animals. Whereas the surveillance activities should be carefully designed to generate statistically sound, unbiased estimates of the national prevalence of AMR, the implementation also needs to be sufficiently practical and cost-effective for the sake of future sustainability.

(Section 2.6.1/Annex 1)

For estimating resistance levels in commensal bacteria

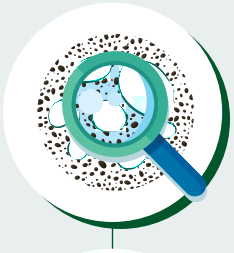
2.6.1 A Sample size calculation for active surveillance of food-borne zoonotic bacteria — **A**

(Section 2.6.2/Annex 2)

For estimating resistance trends in foodborne bacteria

2.6.1 B Sample size calculation for food-borne zoonotic bacteria using isolates already available — **B**

- i. From existing national monitoring programmes for foodborne bacteria
- ii. Existing active surveillance for AST of commensal bacteria
- iii. Other sources



What bacteria will you target? (Section 2.4)
If resources do not allow all of the bacteria mentioned here to be included in the surveillance programme, the following order of priority for inclusion is recommended: (1) *Escherichia coli* (2) *Salmonella* spp. (3) *Enterococcus faecium* and *E. faecalis* (4) *Campylobacter* spp.

What specimen to collect and how? (Section 3.1.1 / 3.2)
Caecal samples are recommended since they generally provide better recovery for most bacterial species of interest.

How should the samples be labelled? (Section 3.3)
The samples should be clearly labelled using permanent marking instruments. If possible, labels should be prepared prior to the sampling. The information should be placed in a plastic envelope on the outside of the shipping container and should always accompany the samples to the laboratory.

How to package and transport samples? (Section 3.4)
In the interest of veterinary public health, animal specimens must be transported safely, timely, efficiently and legally from the place where they are collected to the place where they are analyzed (OIE, 2017). The samples must be kept in a low-temperature environment at all times from the point of collection until they reach the laboratory where the same temperature should be maintained until the samples are processed .

- **Processing of samples upon arrival at the lab (Section 4.1)**
- **Isolation of target bacteria (Section 3.1.1 / 3.2)**
  - 4.2.1 *Salmonella* spp.
  - 4.2.2 *Eschericia* coli.
  - 4.2.3 *Campylobacter* spp.
  - 4.2.4 *Enterococcus* spp.
- **Antimicrobial susceptibility testing (Section 4.3)**
  - 4.3.1 Methods
  - 4.3.2 Interpretation
  - 4.2.3 Quality control
  - 4.2.4 Harmonized panel
- **Storage of bacterial isolates (Section 4.4)**

1.5 Towards regional harmonization and standardization

Although this guideline is largely anchored in international standards, it also underscores that national AMR surveillance plans must be designed in accordance with the country's standing priorities, needs, resource availability and current capacity. Countries are encouraged to commence implementing surveillance at whatever level their current capacity permits, but the aim for all is to move progressively towards the regional targets for harmonization. This regional document provides general guidance and principles as countries initiate and progressively pursue the ideal sampling, sample collection, laboratory methods, and data management relevant to AMR surveillance. Some key areas are highlighted to underscore where regional consistency is desired in the interest of ensuring regional harmonization and standardization on AMR surveillance in the Asia-Pacific region (Figure 1.2). To make optimal use of synergies it is also recommended to integrate surveillance of AMR in livestock with similar efforts in the public health sector.

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Figure 1.2 **Entry points and subsequent targets for regional harmonization on surveillance of AMR in food-borne microorganisms from healthy food animals and their products**

CONVENIENT ENTRY POINTS FOR INITIATION (Note: This is mainly to trigger surveillance initiatives; findings should not be extended to the population and must be interpreted with caution)		TARGETS FOR REGIONAL HARMONIZATION These may be integrated in the planning and design at the outset, or progressively over time as the country progresses in its routine AMR surveillance.
AMR data are obtained from bacteria from the most accessible population of animals.		**TARGET POPULATION** AMR data from bacteria obtained from the known main food-producing animal species and contributing to the most consumption yield in the country are prioritized.
AMR data are obtained from a convenient number of samples and based on accessibility to these animals. Data obtained cannot be extended to the population of interest and should be limited to the samples tested. Information may be used as the basis for planning an expanded surveillance plan.		**SAMPLING STRATEGY** Takes into account both the epidemiologic (e.g. representativeness) and biologic (e.g. type of sample, timing) considerations, as well as the feasibility of logistical support for implementation.
Starts with targeting *E.coli,* considering the available resources and capacity. If there is an operational food-borne zoonoses surveillance programme (e.g. Campylobacter, Salmonella) consider their inclusion but give particular attention to context from which and how and when the isolates were obtained when drawing conclusions.		**TARGET BACTERIA** Both zoonotic (*Salmonella* spp. and *Campylobacter* spp.) and commensal (*E. coli* and *Enterococcus* spp.) bacteria are included in the routine AMR monitoring and surveillance.
Qualitative data are obtained through disk diffusion method with or without consideration of international standards. Value and validity of resulting data may be compromised and will have limited use for an AMR surveillance programme.		**TYPE OF DATA GENERATED** Quantitative data using Minimum inhibitory concentration (MIC) following international standard methods are generated, reported, and stored.
A few select antimicrobials are included in the panel. The appropriate highest priority critically important antimicrobials are preferred.		**PANEL OF ANTIMICROBIALS** The core panel of antimicrobials monitored is harmonized with that of the region.

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CHAPTER

2 SAMPLING FOR SURVEILLANCE OF ANTIMICROBIAL RESISTANCE IN BACTERIA FROM HEALTHY FOOD ANIMALS AND THEIR PRODUCTS

2.1 Introduction

Surveillance of antimicrobial resistance (AMR) in food-borne bacteria from healthy food animals and their products provides an unbiased estimate of the national prevalence of AMR at the farm level (or at retail level) for different bacterial/antimicrobial combinations (i.e. the proportion of farms that have AMR for the given combination). The cumulative data from the surveillance of AMR in food-borne pathogens and commensal bacteria from food animals and their products are accepted as providing meaningful information that should guide evidence-based actions to address AMR. Such information has the potential to transform policies and practices, and should thus be based on accurate data representing the population being addressed. Quality AMR surveillance data starts with proper methods for obtaining samples from which these data will be generated. This chapter discusses the options and methodologies for sampling for the surveillance of AMR in food-borne pathogens and commensal bacteria from food animals and their products.

2.2 Target population

The target population is the population about which conclusions are to be inferred. Once the purpose and objectives of the AMR surveillance have been defined, countries should identify and prioritize their target populations that will best serve their purpose. This prioritization, which can which can comprise a few populations or a large number of populations depending on the resources available, should take into consideration the main food-producing animal species in the country and/or their national consumption. Existing national livestock databases, such as FAO STAT (http://www.fao.org/faostat/en/#data), will be most useful for this purpose.

2.2.1 Target food animal species

Each country should prioritize the main food-producing animal species contributing to food consumption in the country. Poultry (layers and broilers), swine, and cattle are the major sources of animal food and products in the region, and bacterial isolates from these species should thus be targeted depending on the country's preferences. The selection can be expanded as necessary in accordance with the country needs, access to available resources, and value in their desired outcomes for AMR surveillance. The monitoring should primarily focus on domestic animals as domestic production can be linked to antimicrobial use. It is important to note that because this particular type of AMR surveillance is for public health purposes, the isolates must be from healthy animals intended for food consumption. The country may wish to expand to cover clinical isolates but this will be a separate surveillance or monitoring component most likely using a passive approach to sample collection, different sample types, expected results, data interpretation and reporting, and is thus separately addressed and covered under Regional AMR monitoring and surveillance guidelines volume 2 on monitoring and surveillance of AMR in animal pathogens from diseased livestock and poultry.

2.2.2 Target food of animal origin

Countries may also choose to include meat and other food products from the main food-producing animal species and should reflect consumption patterns in the country. As chicken, pork, and beef are the types of meat most commonly consumed in the region, the appropriate meat products should thus be prioritized. The AMR monitoring should primarily focus on target meat domestically produced to assess practices in the country, or representative of consumption sources for assessment of risks.

Recommendations when identifying the target population: member countries should examine their livestock production systems on the basis of available information and assess which sources are likely to contribute most to a potential risk to animal and human health (Section 6.8.4 of OIE, 2018). Countries should prioritize the main food-producing animal species contributing to the most consumption yield in the country.

2.2.3 Target imported food of animal origin

AMR monitoring can also be expanded to imported meat. This is complementary monitoring that should be analyzed and reported separately from the results for domestically-produced meat. Net-importing countries in the region may choose this as their priority for AMR surveillance.


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2.3 Sample sources

Once the target population has been identified, the sampling points for this target population should be determined. These could be directed towards the animals on the farm, live animal markets, or slaughterhouses. Villages could also be used as proxies. Selection will depend on the type of animal production and type of bacteria targeted by the country. Information generated from samples collected at these sources can be integrated with data from other sources, such as data from human isolates to allow integrated surveillance.

2.3.1 Food animals sampled at the farm

Faecal samples of food animals at the farm may be collected to monitor AMR. Although data from isolates obtained at this level of the chain allow more accurate assessment of the impact of antimicrobial exposure of the source animals, representativeness of such samples is often compromised particularly in countries where this is limited by access to private farms, logistic challenges, and/or extensive costs. However, if laying hens are identified as the target population, sampling at the farm level (e.g. using boot swabs) will likely remain the best approach since layers are not routinely slaughtered like broilers. Villages could be used as proxies for farm or slaughterhouse sampling where sampling frames are lacking.

2.3.2 Food animals at slaughter

This is the point where livestock are closest to consumer exposure. It is usually also the most convenient and cost-effective point for collecting animal samples. For most livestock in the region, there is a large number of mostly traditional slaughter places. Unlike in developed countries where slaughtering is much more concentrated in large slaughterhouses, small-to-medium-sized slaughterhouses predominate in the region. This is largely because of prevailing traditional meat-marketing systems where red meat is supplied “hot” or unrefrigerated to consumers. Likewise, for poultry, small-scale slaughter points predominate. These are often makeshift slaughter areas linked with live bird markets. **For consistency and harmonization in the region, it is recommended that all sampling is conducted at slaughter places except** for layer poultry (see Section 2.3.1).

2.3.3 Fresh meat

Monitoring of AMR in meat can also be performed. Fresh meat can be collected either at the carcass cutting plant in slaughterhouses or at retail outlets. The sampling at the carcass cutting plant may make it easier to differentiate between the domestic and imported products. At retail outlets, aside from issues of potential cross-contamination, domestic and imported raw meat, either fresh or frozen, may be mixed and difficult to distinguish based on labelling. This may necessitate additional work and may be prone to errors. However, the sampling at retail outlets will help to better assess consumers’ exposure to resistant bacteria.

Sampling should focus on the animal populations at the production phase that consumers are most likely exposed to, preferably close to or at slaughter. It is also particularly important that the bacterial isolates originate from healthy animals sampled from randomly selected flocks or randomly selected slaughter animals to avoid bias towards a resistant population, as sick animals often get treatment. Regardless of the issues of AMR, sick animals should not enter the food chain.

RECOMMENDATIONS when identifying the source population for this type of surveillance: all countries should aim to conduct AMR monitoring and surveillance targeting animals at the end of their production cycle. This is normally most practical using abattoir sampling but may be done on-farm in some circumstances if it is simpler and cheaper. Surveillance of meat at retail outlets (domestic and/or imported) may be included as an additional option.

2.4 Bacterial species to be monitored

The monitoring of AMR in food animals and meat should cover commensal bacteria and food-borne zoonotic bacteria (Table 2.1).

2.4.1 Commensal bacteria

Commensal bacteria including *Escherichia coli, Enterococcus faecium* and *E. faecalis* are carried by all animals. They are commonly isolated from animal intestinal contents and faeces and relevant to human health. Commensals are exposed to antibiotics taken via feed and/or water, and could thus serve as reservoirs for transferable resistance determinants that may be transferred to other commensal and pathogenic bacteria in the animal or human gut. Most resistance phenotypes present in animal populations are present in commensals. It is more accurate to monitor the effects of antimicrobial use and trends in the prevalence of resistance in commensals than in food-borne pathogens (EFSA, 2018).

2.4.2 Food-borne zoonotic bacteria

Food-borne bacteria (*Salmonella* spp. and *Campylobacter* spp.) (Table 2.1) are bacteria occurring in animals and causing food-borne infections in humans. Human surveillance data on food-borne diseases can be used to set priorities for zoonotic bacteria surveillance. It is expected that the prevalence of zoonotic bacteria may be low or very low in the future as a result of better or successful control programmes. Therefore, commensals are likely to be important in AMR monitoring for better comparison in the future.

Table 2.1 Priority bacterial species to be monitored in the region

Type of bacteria	Example	Notes
Commensal bacteria	*E. coli*	Represents commensal population of Gram-negative bacteria.
	E. faecium and E. faecalis	Represents commensal population of Gram-positive bacteria.
Food-borne zoonotic bacteria	*Salmonella* spp.	Particular patterns of resistance are associated with certain *Salmonella* serovars (EFSA, 2019). Therefore, it is recommended that Salmonella serotyping is performed to allow epidemiological tracing of isolates with particular resistance patterns.
	Campylobacter spp.	The campylobacter strains for antimicrobial susceptibility testing should be identified to the species level. The monitoring is often limited to *C. jejuni* and *C. coli*.

Recommendations when identifying the bacteria to be monitored:
where possible, all four bacteria should be included in the surveillance programme. However, if resources do not allow this, the following order of priority for inclusion is recommended:

1. *Escherichia coli*
2. *Salmonella* spp.
3. *Enterococcus faecium and E. faecalis*
4. *Campylobacter* spp.

If existing surveillance activities for food-borne zoonoses already include pathogens such as *Salmonella* spp., and *Campylobacter,* isolates from these programmes may also be included. However, generated data should be interpreted with caution taking into consideration the sampling methods applied at the outset and possible resulting bias.

The target bacteria may also be recovered from intestinal and/or extra-intestinal specimens from clinical cases (e.g. omphalitis, salpingitis, or fowl typhoid in chickens, colibacillosis or clinical salmonellosis in pigs), but it should be emphasized again that for the purpose of the surveillance described here, sick animals should not be included and samples should be obtained **from clinically healthy animals only.**

2.5 Sampling frame

The sampling frame is a list of sampling units within the target population from which samples can be collected. Each country can choose to develop or identify an appropriate sampling frame for their identified target population. This can be a list of farms, slaughterhouses, or other sources suitable for sampling. Where feasible, **it is ideal to have at least 80 percent of the total target population included in the sampling frame from which the actual samples will be drawn.**

Although it is ideal to have a sampling frame that is exhaustive and includes the entire target population, this is often challenging to achieve, particularly in countries in the region with less developed farm and trade information systems, inaccessible terrains, and/or informal channels of trade. Nevertheless, countries should not delay AMR monitoring and surveillance for this reason. It should be emphasised that: (i) findings and conclusions should be limited to the nature of samples and the sampling strategies taken; and that (ii) statistically valid surveys achieving appropriate representativeness and fulfilling the desired objectives of the AMR surveillance as set out in the plan should continue to be pursued progressively by each and every country.

2.5.1 Sampling frame for farms

The epidemiological unit for broilers is the flock,[1] and for fattening pigs it is the farm. As it is often not possible to obtain an exhaustive list of flocks or farms covering the entire population of interest, the sampling frame should include flocks or farms representing at least 80 percent of the population targeted.

2.5.2 Sampling frame for slaughterhouses

The sampling frame should include slaughter facilities that account for for at least 80 percent of the slaughter population of the food animal species prioritized. A complete list of all slaughter places including the number of animals per animal species slaughtered at each setting per year should be made. The list is made from the latest updated annual data.

2.5.3 Sampling frame for retail outlets[2]

The sampling areas (provinces) should account for at least 80 percent of the national retail outlet population. The number of different meat categories to be sampled is assigned proportionally to the size of the human population in the areas.

2.5.4 When a suitable sampling frame is not available

In this case proxy (or indirect) sampling may be used, e.g. if there is no sampling frame of farms/slaughter points, a list of villages may be used as a proxy. A random sample of villages is selected, and animals sampled from one of the farms/slaughter points found in the village. If no farms/slaughter point is found in the selected village, the next randomly selected village is used.

2.6 Sample size calculation

Various references and tools are available and can be used for sample size calculation (some examples include: http://epitools.ausvet.com.au/content.php?page=SampleSize; http://www.openepi.com/SampleSize/SSPropor.htm) However, **it should be noted that this part of the planning would also benefit from the input of relevant units of the Competent Authority with this area of expertise (e.g. the epidemiology section of the Veterinary Authority).** Whatever approach is used, it should reflect the set objectives for the national AMR surveillance, and comply with the OIE Terrestrial Animal Health Code Section 6.8.4 (OIE, 2018a) which states that:

- "The sample size should be large enough to allow detection or determine prevalence of, or trends in, existing and emerging antimicrobial resistance phenotypes.

[1] **Flock:** All poultry of the same health status kept on the same premises or in the same enclosure (EFSA, 2018).

[2] **Retail outlets** are outlets selling directly to the final consumer for subsequent domestic consumption, i.e. outlets such as supermarkets, specialist shops and markets. This excludes catering activities, restaurants and similar outlets (EFSA, 2008). Chilled meat includes meat that is wrapped, vacuum-wrapped or wrapped in a controlled atmosphere. This could be carcasses or meat portions with skin on from broilers; unskinned carcasses/meat portions for broilers, pork and beef that are typically displayed without skin (EFSA, 2014).

- The sample should avoid bias and be representative of the animal population, process, product or other unit of interest whilst taking into account the expected prevalence of the bacteria in the sample type, the expected prevalence of the resistance phenotype and the desired level of precision and confidence.

- The sample size calculation should be based on independent samples. However, if there is any clustering at the establishment or animal level, the sample size should be adjusted accordingly.

- At low levels of expected prevalence, exact methods of sample size calculation should be preferred to approximate methods.

- Samples from which bacteria were not isolated cannot be used in the calculation of prevalence of the resistance phenotype."

2.6.1 Sample size calculation for estimating resistance levels in commensal bacteria

The commensal intestinal flora is commonly isolated from animal intestinal content and faeces, i.e. the probability of obtaining positive samples can be assumed to be 100 percent. *Escherichia coli* can be used as an indicator for Gram-negative bacteria, whereas *Enterococcus faecium* and *E. faecalis* can be used for Gram-positive bacteria. Most resistance phenotypes circulating in animal populations are present in these species.

These indicator bacteria are important to monitor because: (i) commensal bacteria that contaminate food may also be considered a potential AMR hazard for consumers as they can harbour transferable genes leading to resistance spread; and (ii) the impact of antimicrobial use in the target population, as well as trends in the occurrence of resistance, can be studied in these common indicator bacteria (EFSA, 2012a).

For surveillance targeting commensal bacteria, the aim is to estimate the unbiased national prevalence of AMR at the farm level for different bacterial-antimicrobial combinations (i.e. the proportion of farms that have AMR for the given combination). The **unit of interest is the farm,** as this is the level at which management (antimicrobial treatment) and transmission (mixing of animals) should result in a relatively homogenous AMR profile. For each farm, we need a result for each bacterial/antimicrobial combination (resistant/not resistant or wild type/non-wild type). For an unbiased estimate of AMR prevalence, it is necessary to use representative sampling of epidemiological units. Within epidemiological units, it is assumed that the AMR profile is largely consistent between different bacteria and animals. **Steps and examples for calculating sample size for this approach are shown in Annex 1.**

2.6.2 Sample size calculation for estimating resistance trends in food-borne zoonotic bacteria

Surveillance of AMR in zoonotic food-borne pathogens also provides estimates of the effect of antimicrobial usage in food animals and helps determine trends allowing an assessment of the effectiveness of reduction efforts. Unlike commensal bacteria, however, food-borne pathogens can potentially cause disease in humans, and thus the resistance arising from these organisms presents a more direct link to AMR risk for humans.

(1) Sample size calculation for active surveillance of food-borne zoonotic bacteria

The same approach in Steps 1 to 3 described in Annex 1 for commensal bacteria may be carried out to establish the prevalence of resistance among food-borne pathogen isolates (i.e. *Salmonella* spp. and *Camplyobacter* spp.) based on the expected frequency of these bacteria and the extent of resistance among them. Because these food-borne pathogens are expected to be less commonly isolated, active surveillance for this type of bacteria will require more specimens, and thus, more resources. **Steps and examples for calculating sample size for this approach are presented in Annex 2.**

(2) Sample size calculation for surveillance of food-borne zoonotic bacteria using isolates already available In cases where active surveillance will not be feasible given the limitations in resources, countries may adapt the approach being taken by the European Union (EFSA, 2007; EFSA, 2012a and EFSA, 2014) which used the following assumptions: (i) infinite population size for the number of bacteria isolates in each study population and country; (ii) confidence level of 95 percent and a power of 80 percent; and (iii) perfect sensitivity and specificity of the diagnostic test (AST). Following these assumptions, the target number of isolates for susceptibility testing for each study population (broiler, layer poultry, pigs, cattle, etc.) was set to be **n = 170** which adequately allows:

- the detection of a change of 15 percent in the situation of widespread resistance (50 percent proportion of resistance) and the detection of an increase of 5 percent in the situation of a few pre-existing resistant isolates (0.1 percent proportion of resistance); and

- an accuracy of +/- 8 percent for the purpose of determining a proportion of resistance in the worst-case scenario of 50 percent resistant isolates.

When a linear trend exists within a country, smaller changes in proportion can be detected over time. In the case of three years continuous monitoring:

- starting from an initial proportion of resistance of 50 percent, a 5 percent decrease in proportion of resistance per year can be detected; and

- starting from an initial proportion of resistance of 0.1 percent, an increase by 2 percent per year can be detected.

As for surveillance of AMR in commensal bacteria, the conditions for developing the sampling plan (Section 2.7) should be taken into consideration for food-borne zoonotic bacteria (e.g. isolates should come from healthy animals intended for consumption). Where conditions are not satisfied or where limitations exist (e.g. non-random sampling instead of random sampling), these should be recorded and taken into consideration when results are interpreted.

For this purpose, possible sources of isolates to be tested could be:

(i) The use of existing national monitoring programmes for food-borne bacteria

Some countries have existing surveillance programmes for *Salmonella* spp. and *Campylobacter* spp. which can be sources of the isolates to be tested. It should be noted however that this should not be interpreted as an estimate of prevalence of resistance given the sample size and the nature of sampling design for food-borne pathogen surveillance which is often risk-based rather than random. It is recommended to focus on *Salmonella* serovars of clinical importance, i.e. *S. Typhimurium* and *S. Enteritidis* (OIE, 2018a and OIE, 2018b).

(ii) The use of existing active surveillance for AST of commensal bacteria

Isolates may also be derived from specimens intended for active surveillance of commensal bacteria, noting that unless prevalence of the target food-borne pathogen is at least 45 percent, the expected number of recovered

isolates will not be sufficient to reach the desired number of isolates for this purpose and thus a bigger sample size should be considered.

(iii) The use of other sources
Routine testing from food establishments, passive laboratory surveillance, research, and other sources of isolates may be carried out, but the considerations for designing a sampling plan as described in Section 2.7 should be taken into account, and where the sampling is limited, the nature of the sampling should be taken into consideration when interpreting the results.

2.7 Sampling considerations

There are various considerations in the design of surveillance and monitoring of AMR in food-borne bacteria from healthy animals. The surveillance activities should be carefully designed to generate a statistically sound, unbiased estimate of national prevalence of AMR, but the implementation also needs to be sufficiently practical and cost-effective if such activities are to be sustainable. The considerations that need to be underscored in the design development are shown in Figure 2.1

Figure 2.1 Main considerations in the design of surveillance and monitoring of AMR in food-borne bacteria from healthy animals

2.7.1 Health status of the animal sources

Because this particular type of AMR surveillance is focused on the prevalence of AMR at the human-animal consumption interface, samples should come from healthy animals. Sick animals should not be part of this surveillance component (and should not enter the food chain).

2.7.2 Emphasis on domestic production

This is particularly important if the AMR surveillance data is primarily intended for policies and recommendations on antimicrobial usage in the country. If the main intention is to measure and understand the risks of AMR to the human population relevant to their exposure to foods of animal origin, this should include exposure to imported products and should thus be reflected accordingly in the sampling plan. Results of the surveillance can be put in context with the extent to which antimicrobial resistance is found in people.

2.7.3 Sampling by probability proportional to size

The number of samples collected in each stratum (e.g. farms, slaughterhouses, retail outlets, or geographic location) should be proportional to the size of the respective stratum in the sampling frame. This allows better representation of the strata as these are represented in accordance with their proportional share in the overall population.

2.7.4 Sampling frequency

The sampling is performed on an annual basis with – ideally – equal distribution over the year. Distributing the collection throughout the year enables different seasons to be covered, allows spread of demand for manpower, and also helps in having better work traffic from the field to the laboratory. However, if production is seasonal with important peaks, this should be considered in the design. As the national surveillance plan is likely to include more than one livestock species, a sampling interval of two or three years may be considered for each study population in order to optimize the use of resources in the case where they are too limited to cover all species each year.

2.7.5 Ensuring isolate representativeness/ uniqueness and avoiding sample duplication

What is being measured is the different bacterial-antimicrobial combinations (i.e. the proportion of farms that have AMR for the given combination) within the unit of interest (farm or flock) where exposure (antimicrobial treatment) and transmission (mixing of animals) takes place. It is assumed that the AMR profile is largely consistent in the same organisms in the same epidemiological unit. Thus, obtaining multiple, non-unique isolates from the same epidemiological unit may lead to distorted information and misleading interpretation. To avoid this, the sampling plan for each target organism should ensure that the considerations identified in Table 2.2 are taken into account when selecting recovered target isolates for AST.

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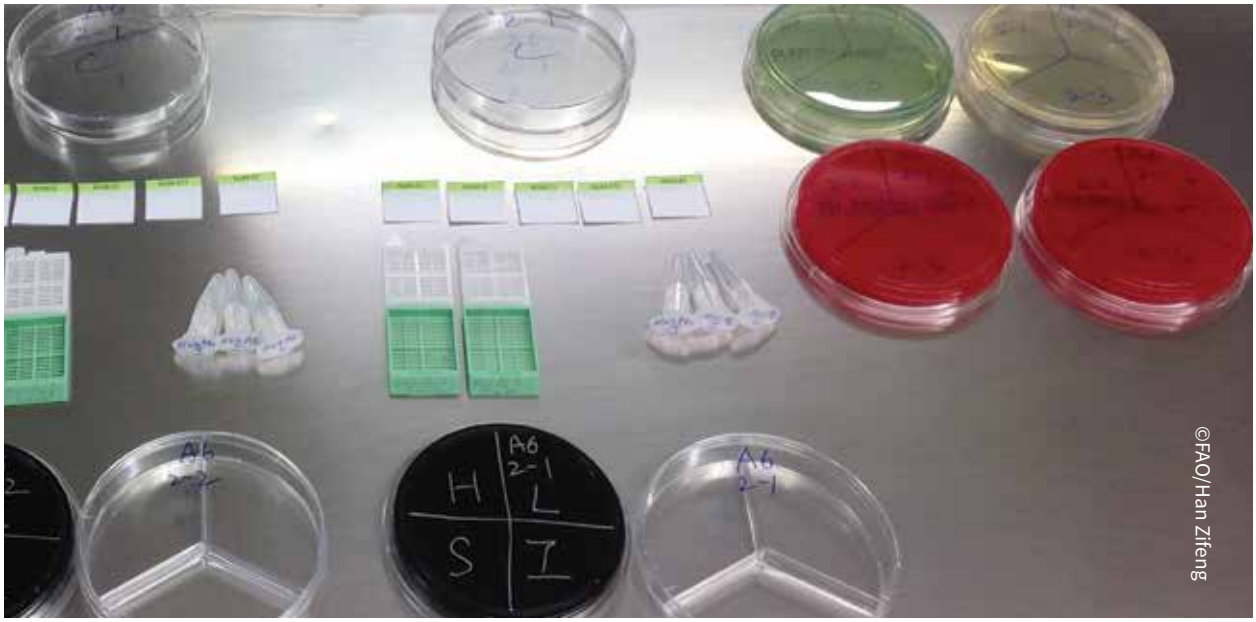

Table 2.2 Sampling plan for bacterial species

Target isolates	Considerations to reflect representativeness
Salmonella spp.	No more than one isolate per Salmonella serovar from the same epidemiological unit per year. Salmonella isolates of the same serovar from the same epidemiological unit are expected to have similar resistance patterns.
Campylobacter coli and jejuni	Only one isolate/bacterial species from the same epidemiological unit per year.
E. coli	Only one isolate from the same epidemiological unit per year.
E. faecium and E. faecalis	Only one isolate/bacterial species from the same epidemiological unit per year.

2.7.6 Streamlining of respective mandates

In the interest of efficiency, planning should also factor in the respective mandates of involved agencies where relevant. This will allow the steps to be integrated into the normal routine operations within the system, and help make the overarching efforts more relevant across the animal health sector.

2.7.7 Non-random sources in monitoring and surveillance of antimicrobial resistance

Although population-based AMR surveillance regularly conducted over time allows analysis of AMR trends, a wide variety of non-random surveillance sources may also be available (or specifically obtained) to complement this work. This includes AMR data from isolates recovered from routine testing/screening, private sector data, sentinel herds or flocks, research studies and other potential AMR data sources. **It should be noted, however, that the non-random data generated from such approaches should be viewed with caution as these do not necessarily represent the target population and if used to analyze AMR trends need to be interpreted with caution.** Furthermore, such data should be generated using harmonized laboratory protocols.

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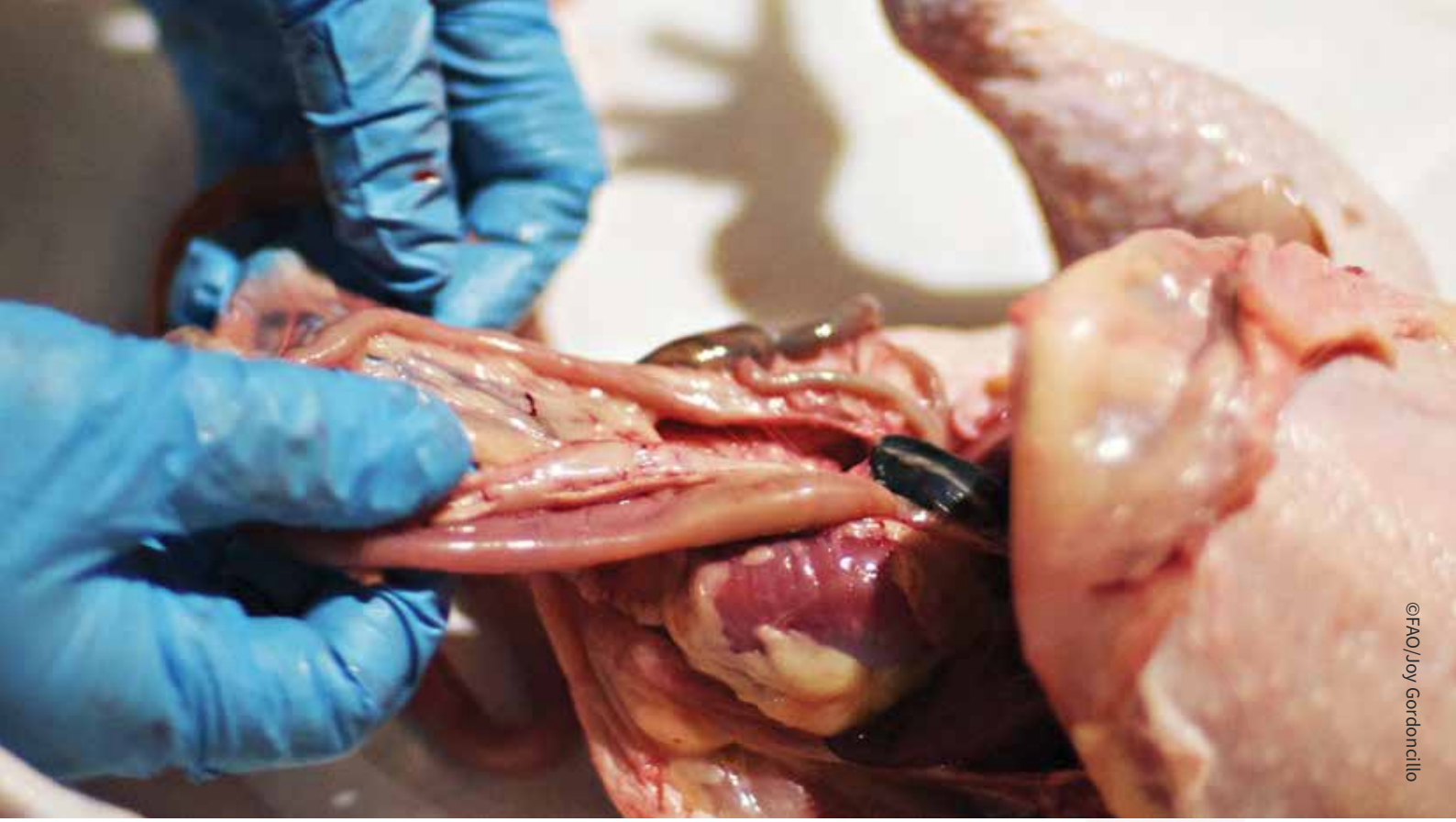

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CHAPTER

3 SAMPLE COLLECTION AND TRANSPORT

3.1 Introduction

In addition to the considerations that need to be made on the statistical basis for sampling for AMR monitoring and surveillance, emphasis should also be placed on the quality of the specimens to be collected, transported and processed at the laboratory. The appropriateness and quality of samples – sustained from field collection to laboratory processing – will contribute to building a quality data set and body of information that will address objectives as set by the country.

3.1.1 Samples to collect for animal–bacteria combinations

As a starting point, countries may consider the recommended combination of zoonotic agents (i.e. Salmonella and Campylobacter) and commensal bacteria (i.e. E. coli and Enterococcus) in food animals and fresh meat noting that the type of samples collected will depend on the animal species that these will be collected from and the target bacterial species. Other samples that could be used and the recommended samples for animal–bacteria combinations targeted are presented in Table 3.1.

Table 3.1 Recommended combination of sampling point, bacterial species and sample type for surveillance of AMR in different livestock species and in meat[a]

Animals	Place of sample collection	Bacterial species[b]					
		Salmonella	*E. coli*	*C. coli*	*C. jejuni*	*E. faecium*	*E. faecalis*
Broiler	Farm	Boot swab	-	-	-	-	-
	Slaughterhouse	Caecum	Caecum	Caecum	Caecum	Caecum	Caecum
Layer	Farm	Boot swab	-	-	-	-	-
Pigs	Slaughterhouse	Caecum[d]	Caecum	Caecum	-	Caecum	Caecum
Cattle	Slaughterhouse	Caecum[d]	Caecum	-[c]	-[c]	Caecum	Caecum
Chicken meat	Slaughterhouse, retail outlet	Skin	?	Skin	Skin	?	?
Pork	Slaughterhouse, retail outlet	Meat	Meat	Meat	-	Meat	Meat
Beef	Slaughterhouse, retail outlet	Meat	Meat	-	-	Meat	Meat

[a] Country experiences should be taken into account when developing an AMR surveillance plan as they may vary. In Japan, for example, although Salmonella in chicken can be detected, there is a low detection rate in cattle and pigs. The Japanese also recover *Campylobacter coli* from pig at slaughter, and *C. jejuni,* from cattle and broilers.
[b] Should be tested (if possible).
[c] Prevalence of *C. coli* and *C. jejuni* is very low.
[d] Rectal swabs can also be used, ensuring that samples do not come in contact with the environment and that sufficient weight is collected for bacterial isolation.

As explained in Section 2.3.2, countries should aim to conduct AMR surveillance targeting animals at the end of their production cycle, which is most conveniently addressed by targeting abattoirs. This is also the most cost-effective option. Caecal samples are recommended since they generally provide better recovery for most bacterial species of interest. Caeca also better reflect the farm-level exposure to antimicrobials as compared to meat or other specimens (e.g. cloacal or faecal samples), which also present the risk of being contaminated with environmental microorganisms. An exception is for layer poultry, where boot swabs are the preferred means if looking into slaughtered layer hens at abattoirs is not feasible or preferred.

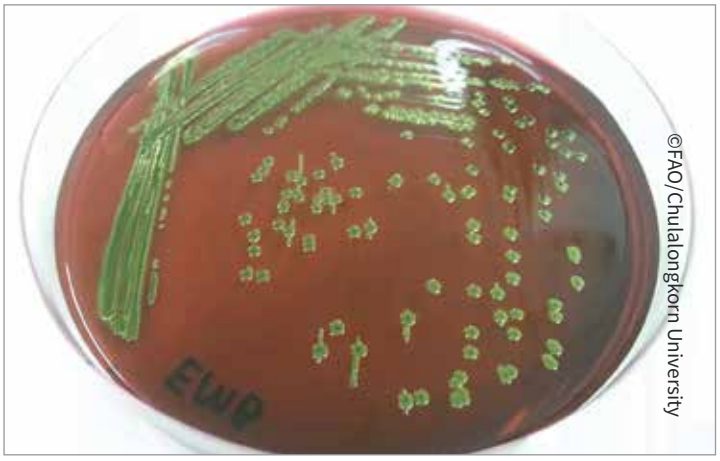
3.2 Sample collection

Careful consideration must be given to the collection, containment, and storage of the specimens, including biosafety measures that must be in place to prevent contamination of the environment or exposure of other animals and humans to potentially infectious materials (OIE, 2017). It is important that only staff well trained in standard sampling procedures perform sample collection. Sterile techniques need to be applied for sample collection and samples need appropriate storage to avoid contamination.

3.2.1 Collection of samples at slaughter

Caecum from broilers

Each batch of broilers is assumed to represent a group of chickens raised together in one shed, and having experienced the same antimicrobial exposure. Thus, it is critically important to consider that each batch, unless clearly indicated otherwise, is a single sample source and should be treated as such.



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For broilers, it is more practical to collect whole intact caeca. Samples are randomly or systematically taken from healthy animals, if possible within ten minutes of slaughter. It is important to make sure that the caecum is intact and full. Pooling of samples from the same batch may be done as necessary; if done, one pooled sample comprises the intact caecal contents of birds from the same slaughter batch that is assumed to have originated from the same unit of interest (farm). Individual or pooled caeca collected are placed in a single sterile plastic bag or jar that will be used in the bacterial isolation step.

Broiler carcasses

The caecum and whole carcass must be from the same slaughter batch. Each pooled sample originates from a different holding or flock to avoid clustering. In the slaughterhouse, one whole carcass is collected immediately after chilling but before cutting, freezing or packaging (EFSA, 2012a). Each sample collected is placed in an individual sterile plastic bag. The neck skin and breast skin from the whole carcass collected are used for examination of Salmonella and Campylobacter.

Caecum from pigs

The sample of caecal content may be derived from one carcass (single sample) or a number of carcasses (pooled sample) per batch/lot of carcasses originating from the same herd (epidemiologic unit).

Caecum from cattle

Faecal/caecal contents are taken from the colon or rectum after incision from one carcass (single sample) or a number of carcasses (pooled sample) per batch/lot of carcasses originating from the same herd (epidemiologic unit). A single sample from one randomly selected animal may be taken. Ensure that a sufficient amount of material for bacterial isolation is taken (See Section 4.1.1)

3.2.2 Collection of samples from the farm using boot swabs

In broiler and laying hen farms, faecal material equivalent to about 300 individual faeces of 1 g should be collected to maximize sensitivity

Preparation of boot swabs:

- Boot swabs are absorptive paper/fabric overboots that are commercially available. Before putting on the boot swab, the surface of the boot swab must be moistened with sterile recovery diluent (e.g. maximum recovery diluent (MRD) containing 0.8 percent sodium chloride, 0.1 percent peptone in sterile deionised water).

  Boot swabs can be moistened as follows: (i) recovery diluent can be poured inside each boot swab before putting on over the plastic overboots; (ii) a boot swab pack can be made by putting boot swabs and sterile recovery diluent in autoclave bags; and (iii) recovery diluent can be sprayed after boots are put on (EC, 2005).

- Once sampling in the chosen sector is completed, carefully remove the boot swabs by inverting the boot swabs to retain materials and place in sterile bag or jar. The bags or jars can subsequently be used for culture of the sample.

- A pair of new plastic overboots should be put on before putting on the boot swabs.

of sampling. This can be achieved by taking five pooled samples of boot swabs in any selected flock. Each pooled sample comprises faecal material fixed to a pair of boot swabs. The samples should be collected in the area inside the house, including littered and non-littered areas but not any outdoor areas in free-range flocks. The floor area of the house is divided into five equal sectors for sampling. The staff walks at least 100 steps per pair of boot swabs within the chosen sector to ensure that all parts of the sector are sampled, including littered and non-littered areas but not any outdoor areas. Once the sampling is completed in each chosen sector, the boot swabs are carefully removed. The boot swabs can be inverted to retain material and placed in a sterile plastic bag or jar that can be used in the bacterial isolation step.

3.3 Sample labelling

The samples should be clearly labelled using a permanent marking pen. If possible, labels should be prepared prior to the sampling. The information should be placed in a plastic envelope on the outside of the shipping container and should always accompany the samples to the laboratory. The microbiology laboratories should record the data of the sampling. Examples of sampling information are presented in Table 3.2 and Table 3.3.

Table 3.2 Examples[a] of sample information collected from farm

Details	Information
Sender/Sample collector details	
Name	
Contact details (mobile/e-mail/telephone/fax numbers)	
Sample collection details	
Date and time of sampling	
Type of farm	
Location/geographical origin (GIS data where available)	
Farm identifying number	
Farm size	
Production category/type	
Sample details	
Sample ID number	
Animal species	
Type of sample	
Pooled (if yes, number of samples in this pool)	
Transport media, if used	
Animal factors (e.g. breed, age, condition, health status, identification, sex)	
Antibiotic history for the past year	

[a] These are just examples and should be modified according to the planned data management strategy of the country. For further details on this, please refer to Section 5.1

Table 3.3 Examples[a] of sample information collected from slaughterhouse/slaughterpoint

Details	Information
Sender/Sample collector details	
Name	
Contact details (mobile/e-mail/telephone/fax numbers)	
Sample collection details	
Date and time of sampling	
Type of slaughterhouse	
Location/geographical origin (GIS data where available)	
Slaughter place identifying number	
Average daily slaughter volume	
Sample details	
Sample ID number	
Animal species	
Type of sample	
Pooled (if yes, number of samples in this pool)	
Transport media, if used	
Animal factors (e.g. breed, age, condition, health status, identification, sex)	

[a] These are just examples and should be modified according to the planned data management strategy of the country. For further details on this, please refer to Section 5.1

3.4 Packaging and transport of the samples

In the interest of veterinary public health, animal specimens must be transported safely, timely, efficiently and legally from the place where they are collected to the place where they are analyzed (OIE, 2017). The samples must be kept in a low-temperature environment at all times from the point of collection until it reaches the laboratory where the same should be maintained until these are processed.

3.4.1 Packaging of the samples

Samples should be placed in appropriate secure containers for transport. Normally, well-packed samples should be placed in cool boxes together with frozen gel packs (temperature below 10 °C). Never freeze the samples as it may kill bacteria. The following should be carefully noted:

- All specimens should be packaged and transported in accordance with local and/or national regulations.
- The procedures should minimize the risk of exposure for those engaged in transportation and should protect the environment and susceptible animal populations from potential exposures.
- Specimens should always be packaged and transported to protect the integrity of the specimens, as well as to avoid cross-contaminating other specimens.

- Minimal requirements for the transport of specimens follow the principle of triple packaging, consisting of three layers: a watertight and leakproof primary inner receptacle, a durable, watertight, leakproof secondary packaging that will protect the primary packaging, and a sturdy outer packaging that will protect the two layers against physical damage while in transit.

3.4.2 Transporting the samples

The samples should be transported immediately or within 24 hours. The laboratory analysis should begin as soon as possible. Information on the time between sample collection, storage in the laboratory and processing should always be recorded. This is especially important when the recommended times are not possible (e.g. isolated sampling sites).

Caecal or faecal samples

These samples should be transported immediately and arrive at the laboratory within 24 hours after collection. The analysis should be performed immediately. If this cannot be managed, the samples should be stored at 4 °C to 7 °C or kept refrigerated and analyzed no later than 72 hours after sampling.

Carcass or meat samples

The sample should be kept at 2 °C to 8 °C. Each plastic bag containing the individual sample should be placed in cardboard or foam boxes together with frozen gel packs. The samples should be shipped to the laboratory on the same day they were collected and arrive at the laboratory within 24 hours after collection. If this cannot be arranged, a transport duration of two days is still acceptable. It is important to ensure that the samples will arrive the laboratory no more than three days after sampling, but if possible this should be avoided. The sample should be stored at 4 °C after the arrival. The analysis should be performed within 72 hours from the time of sampling.

Boot swabs

Boot swab samples should be transported within the same day of sampling and arrive at the laboratory within 24 hours after collection. At the laboratory, samples should be kept refrigerated (4 °C to 7 °C) until examination. The laboratory analyses should be carried out within 48 hours after receipt or 72 hours after collection (EC, 2005).

Campylobacter spp

Campylobacter spp. are sensitive to environmental conditions, including dehydration, atmospheric oxygen, sunlight and elevated temperature. Transport to the laboratory and subsequent processing should therefore be as rapid as possible, preferably the same day, but within a maximum of three days. The samples must be protected from light, extreme temperatures and desiccation. No recommendation on the ideal temperature for transportation can be made, but it is clear that freezing or high temperatures can reduce viability. High temperatures (>20 °C), low temperatures and fluctuations in temperature must be avoided. When the time between sampling and processing is longer than 48 hours, storage at 4 °C (±2 °C) is advised (OIE, 2016).

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CHAPTER

4 LABORATORY METHODS

4.1 Processing samples upon arrival at the laboratory

Once the samples have been brought to the laboratory they are processed to isolate and identify target bacteria. Thereafter they undergo quality controlled antimicrobial susceptibility testing (AST) and the results are interpreted. These activities are all described in some detail in the following sections.

4.1.1 Processing caecal contents

When processing caecal contents in the laboratory for isolation and identification of target bacteria, 28 g of samples are needed to perform analyses for *Salmonella* spp., *Campylobacter* spp., *E. coli* and *Enterococcus* spp. from one sample in parallel. The 28 g portion of the sample is transferred to 252 ml of buffered peptone water (BPW) at room temperature. The mixture is then treated in a stomacher for one minute. This initial suspension shall be used as identified in Table 4.1.

Table 4.1 Distribution of initial suspension (28 g)

Volume	Utilization	Related sections
250 ml (~25 g)	For the detection of *Salmonella* spp.	Section 4.2.1
10 ml (~1 g)[a]	For detection of *E.coli*	Section 4.2.2
10 ml (~1 g)[a]	For detection of *Campylobacter* spp.	Section 4.2.3
10 ml (~1 g)	For the detection of *Enterococcus* spp.	Section 4.2.4

[a] Direct plating can also be done for *E.coli* and *Campylobacter* spp.

It should be noted that the objective is not to determine the prevalence of these organisms, but to obtain representative farm isolates that will be the basis for establishing the prevalence of resistance in particular bacteria–antibiotic combinations. Depending on their relevant capacities, countries may opt to start with at least one organism and expand as circumstances allow. Isolates from ongoing surveillance for food-borne pathogens may also be used, with results interpreted with caution (See Section 2.6.2).

4.1.2 Processing whole broiler carcasses in the laboratory

Using aseptic techniques, the neck skin (if present) and the skin from one side of the carcass (breast skin) is removed to make a 28 g test portion and then it is transferred to 252 ml of room temperature BPW. The sample is then placed into a stomacher bag. Any fat should be avoided (EFSA, 2010).

4.1.3 Processing boot/sock swabs in the laboratory for Salmonella isolation

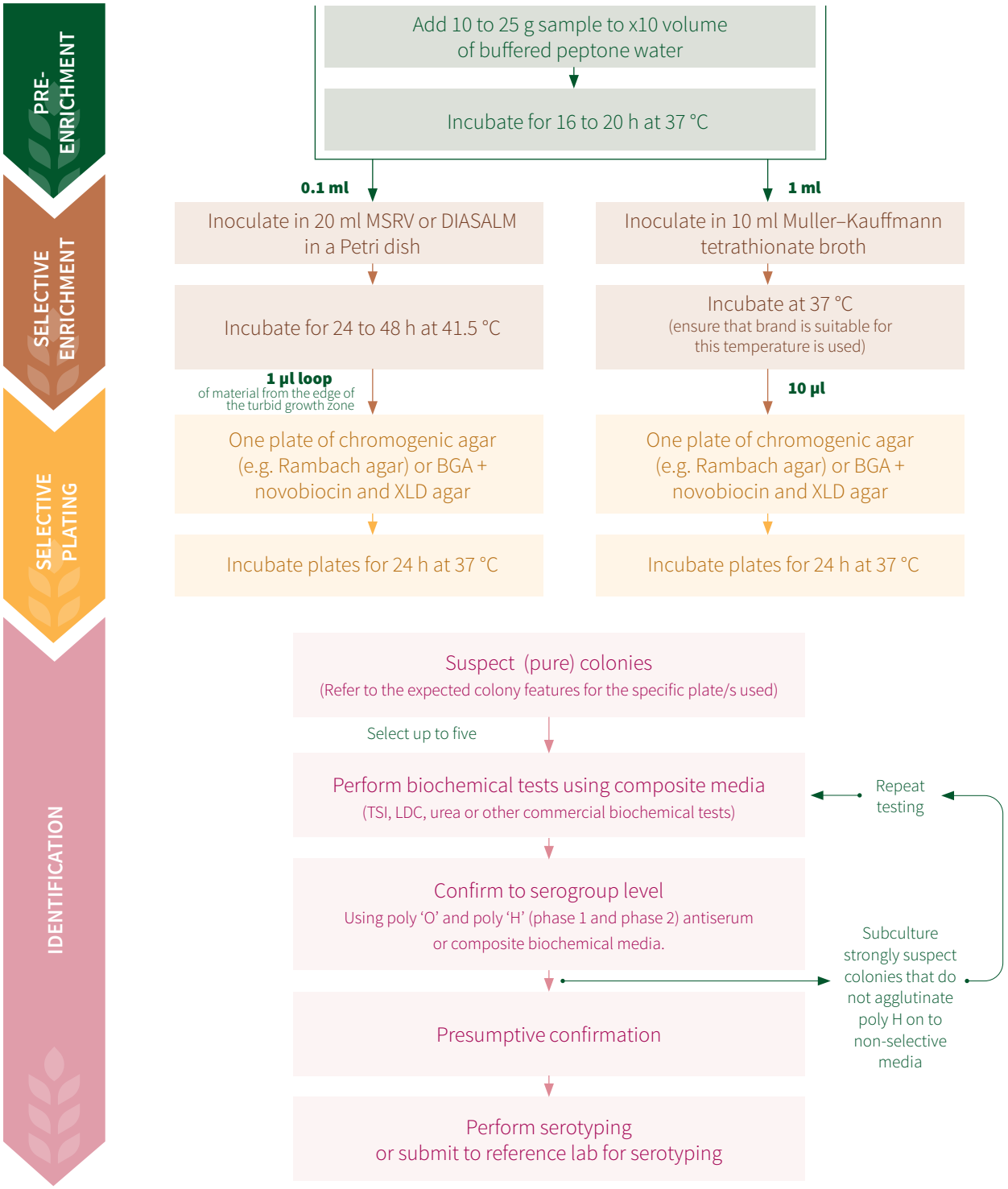
If possible, boot swabs should be gathered at farm level and placed in sterile bags or jars that can be subsequently used for bacteria isolation.The outside of the bag or jar is first sterilized by spraying with 70 percent alcohol. If swabs are transported in bags, carefully evert the bags so that the boot swabs and any loose litter material are emptied into a jar containing 225 ml of BPW along with one litre consisting of pancreatic digest of casein 10 g, sodium chloride 5 g, disodium phosphate 3.5 g, and monopotassium phosphate 1.5 g, pH 7.0. The bag/jar is gently swirled and then placed in the incubator.

4.2 Bacteria isolation methods

4.2.1 Isolation and serotyping of *Salmonella* spp.

There are numerous methods for isolation and detection of Salmonella used worldwide, but the increasing application of external quality assurance programmes has led to greater use of international standard methods. The ISO standard used for Salmonella isolation is the ISO 6579-1:2017 *Microbiology of the food chain – Horizontal method for the detection, enumeration and serotyping of Salmonella spp. Part 1: Detection of Salmonella spp.* Alternatively, the methods for isolation of Salmonella from food, feedstuffs, faecal and environmental samples as outlined in OIE (2016) may be followed as shown in Figure 4.1.

Figure 4.1 Procedures for isolation of Salmonella from food, feedstuffs, faecal and environmental samples



Source: OIE (2016)

At least three isolates from each positive sample should be typed for their serotypes following the Kaufmann–White scheme (Popoff and LeMinor, 1997) or ISO 6579-3:2014 *Microbiology of the food chain – Horizontal method for the detection, enumeration and serotyping of Salmonella* spp. *Part 3: Guidelines for sertyping of Salmonella* spp.

4.2.2 Isolation and identification of *Escherichia coli*

(1) Method 1: Enrichment inoculation

If the number of *E. coli* in a sample is expected to be low, such as when sampling meat from supermarkets or from high-end brands, enrichment should be performed. One gram of sample is mixed with BPW (1/10) and incubated at 37 °C ± 1°C for 18 h to 22 h. Alternatively, inoculation and incubation in selective media can be performed (see ISO 7251-2005 *Microbiology of food and animal feeding stuffs – Horizontal method for the detection and enumeration of presumptive Escherichia Coli – Most probable number technique.).*

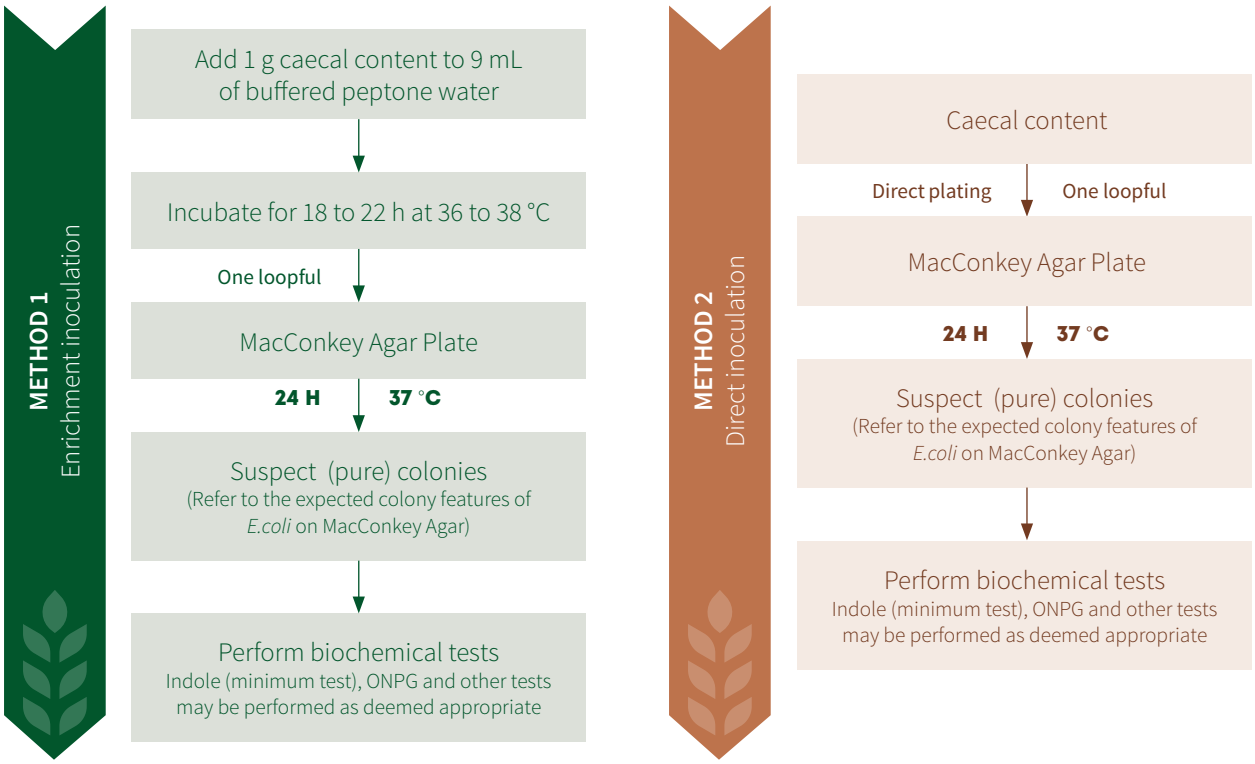
One loopful (10 µl loop) of the overnight culture is applied by streaking onto a MacConkey agar plate. Several typical colonies should be streaked on Eosin Methylene Blue agar (see BAM 4, available at https://www.fda.gov/food/laboratory-methods-food/bam-4-enumeration-escherichia-coli-and-coliform-bacteria) and typical colonies are selected for confirmation by biochemical tests.

(2) Method 2: Direct inoculation

As selective enrichment may enhance growth of a subpopulation that do not represent the *E. coli* population within a tested sample, only a direct inoculation of samples on differentiating media (e.g. MacConkey agar) can be performed (EFSA, 2008). This can be done if the expected number of *E. coli* in a sample (e.g. caecal content) is high. Several typical colonies may be streaked on Eosin Methylene Blue agar and typical colonies are selected for biochemical testing to identify colonies to species level. The minimum requirement is to test for indole production for verification of the species. The ortho-nitrophenyl-β-D-galact opyranoside test (ONPG) can be additionally used to verify presumed *E. coli* isolates. Other confirmation tests can be conducted as appropriate.

The steps taken to perform enrichment inoculation and direct inoculation are shown in Figure 4.2.

Figure 4.2 Basic methods for the detection and identification of *E. coli*

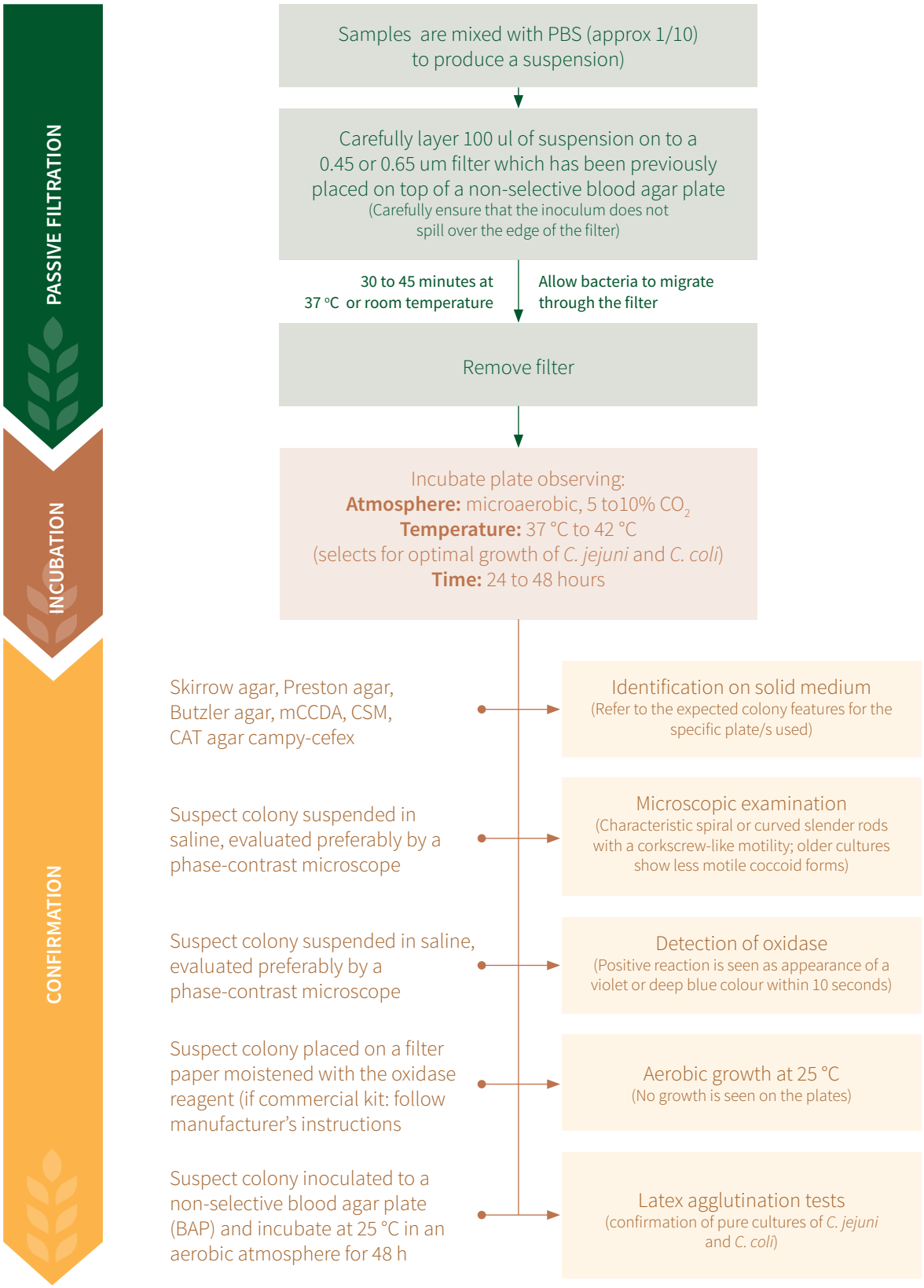


4.2.3 Isolation and identification of *Campylobacter* spp.

Campylobacter jejuni and *C. coli* are thermophilic, Gram-negative, highly motile bacteria that for optimal growth require a microaerobic environment and incubation temperatures of 37 °C to 42 °C. Isolation and confirmation of Campylobacter in caecal content and on the broiler carcass samples should be undertaken as described in ISO 10272-1:2017 *Microbiology of the food chain–Horizontal method for detection and enumeration of Campylobacter* spp. *– Part 1: Detection method.* At least three Campylobacter isolates must be differentiated using phenotypic methods as described in ISO 10272-1:2006(E) *Microbiology of food and animal feeding stuffs – Horizontal method for detection and enumeration of Campylobacter* spp. *– Part 1: Detection method,* or using published molecular methods, e.g. Polymerase Chain Reaction (PCR) techniques.

Direct culture or inoculation of caecal content on a selective medium is also suggested (See Section 4.2.2). Alternatively, isolation and confirmation procedures for *Campylobacter* spp. as outlined in OIE (2016) may be followed. Samples can be plated on selective media (blood or charcoal based media) or the filtration method on non-selective agar can be used (see Figure 4.3). Identification of Campylobacter to the species level will require molecular tests (e.g. polymerase chain reaction (PCR)) or matrix-assisted laser desorption/ionization-time of flight (MALDI-TOF) mass spectrometry.

Figure 4.3 Isolation and confirmation of Campylobacter

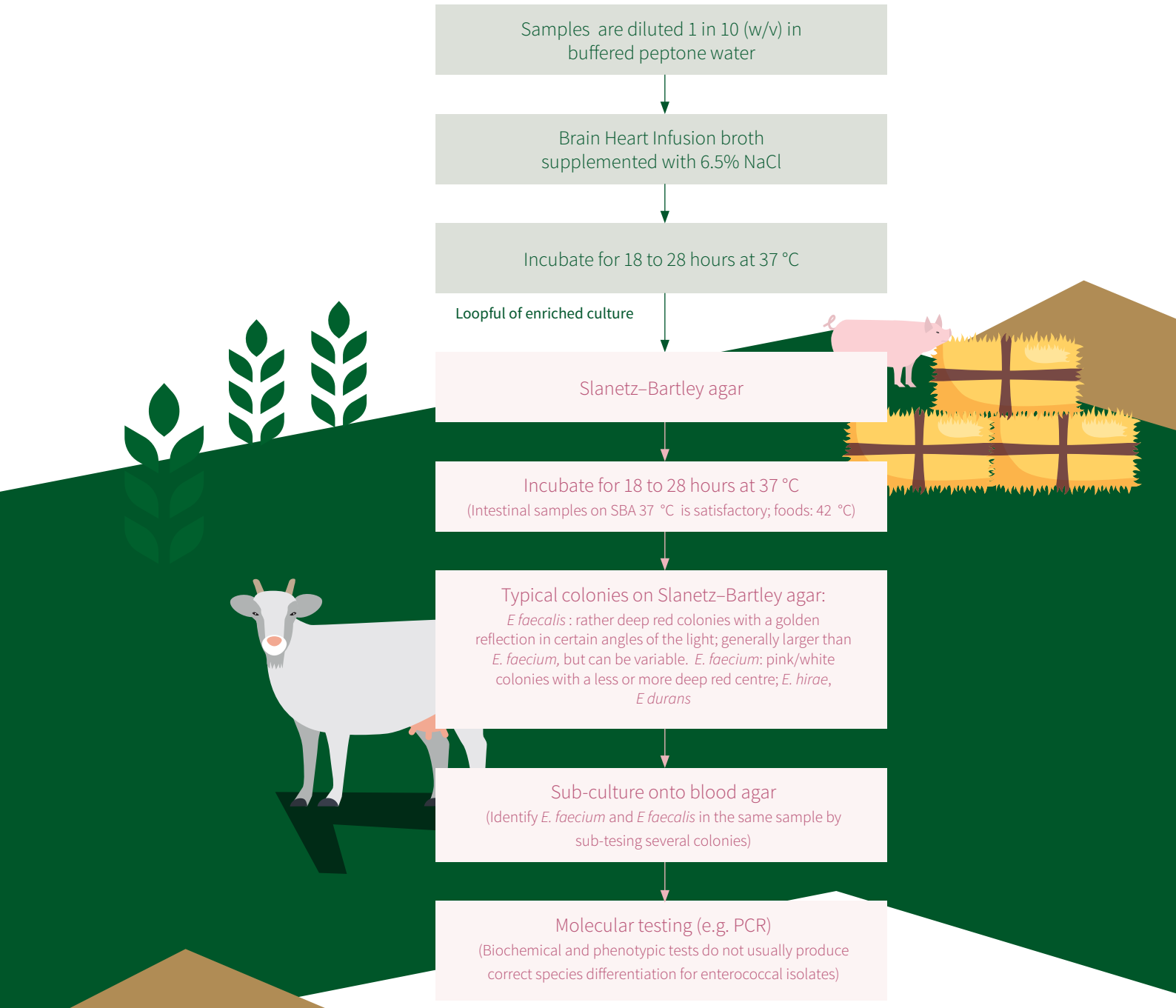


Source: OIE (2016)

4.2.4 Isolation and identification of *Enterococcus* spp.

Different methods can be used for primary isolation of *Enterococcus* spp. A number of different species of Enterococcus can be found as commensals in the gastro-intestinal tract of domestic animals. The use of enrichment broth, as in Figure 4.4, may reduce the sample size required, and is therefore recommended. The recommended method to maximize recovery of *E. faecium* and *E. faecalis* from caecal contents is shown in Figure 4.4 .

Figure 4.4 Detection and identification of *Enterococcus* spp.



Source: EFSA (2008)

Biochemical and phenotypic tests do not usually produce correct species differentiation for all *E. faecium* and *E. faecalis* isolates. Therefore, molecular testing (e.g. PCR techniques) should be applied to confirm the identity of *E. faecium* and *E. faecalis.* The PCR primer sets commonly used are shown in Table 4.2.

Table 4.2 Polymerase chain reaction primer sets for species identification of *Enterococcus* spp.

Primer set	Species	Product	Primer sequences
Primer set 1[a]	*E. faecalis*	941 bp	Forward primer E1: ATCAAGTACAGTTAGTCTT Reverse primer E2: ACGATTCAAAGCTAACTG
	E. faecium	550 bp	Forward primer F1: GCAAGGCTTAGAGA Reverse primer F2: CATCGTGTAAGCTAACTTC
Primer set 2[b]	*E. faecalis*	360 bp	Forward primer FL1: ACTTATGTGACTAACTTAACC Reverse primer FL2: TAATGGTGAATCTTGGTTTGG
	E. faecium	215 bp	Forward primer FM1: GAAAAAACAATAGAAGAATTAT Reverse primer FM2: TGCTTTTTTGAATTCTTCTTTA

[a] The primer sequences for *E. faecalis* and *E. faecium* (Dutka-Malen, Evers and Courvali, 1995)
[b]The primer sequences for *E. faecalis* and *E. faecium* (Jackson, Fedorka-Cray and Barrett, 2004)

4.3 Antimicrobial susceptibility testing (AST)

There are various methods for *in vitro* AST (disc diffusion, e-test, agar dilution, broth microdilution, and broth macrodilution). Whichever method is used, the tests have to be performed in accordance with internationally accepted procedures such as those published by the Clinical and Laboratory Standards Institute (CLSI) or the European Committee on Antimicrobial Susceptibility Testing (EUCAST). It is essential that AST methods provide reproducible results in day-to-day laboratory use and that the data be comparable with those results obtained by an acknowledged "gold standard" reference method.

4.3.1 Antimicrobial susceptibility testing methods

The main methods used are disk diffusion, dilutional susceptibility testing and molecular methods.

(1) Disk diffusion

Disk diffusion refers to the diffusion of an antimicrobial agent of a specified concentration from disks, tablets or strips, into the specific solid culture medium that has been seeded with the selected inoculum isolated in a pure culture (OIE, 2012). Although this is technically more simple to perform, less expensive, and useful for guiding treatment in a clinical setting, this procedure only provides categorical information (susceptible, intermediate, or resistant) and has limited application in AMR monitoring and surveillance (EFSA, 2007; EFSA, 2008; and EFSA, 2019). The disk diffusion method also does not guarantee reproducibility of results for *Campylobacter* spp., or for testing large molecules such as colistin. It should also be noted that whereas zone diameters correlate inversely with minimum inhibitory concentration (MIC) breakpoints, regression line analysis should not be used to extrapolate MIC values from measurements of zones of inhibition because in many cases, although this may be mathematically correct, the relationship cannot be considered comparable to an MIC derived by actual dilution testing for a given isolate (CLSI, 2013). In countries where capacities may be limited to this procedure, interpretation of surveillance results should take such limitation into consideration, and progress towards quantitative methods should be pursued.

(2) Dilutional susceptibility testing

Dilutional susceptibility testing, such as broth or agar dilution techniques, quantitatively measure the *in vitro* activity of an antimicrobial agent against a given bacterial culture by determining the lowest concentration of the assayed antimicrobial agent inhibiting the visible growth of the bacterium being tested. These procedures provide quantitative MIC data, usually expressed in µg/ml, from which clinical breakpoints and epidemiological cut-off values may be derived.

(3) Molecular methods

Molecular methods, in addition to phenotypic methods, may also be utilized for their advantages in speed and accuracy in detecting the underlying genetic determinants of AMR. Some examples of methods include PCR, DNA microarray, whole-genome sequencing (WGS) and metagenomics, and matrix-assisted laser desorption ionization–time of flight (MALDI-TOF) mass spectrometry. It is foreseen that WGS will have a bigger role in the future of AMR surveillance globally. Although essential capacity and facilities exist for AMR-genotypic monitoring in some countries, the larger part of the region is still not ready for this. Additionally, measures such as epidemiological cut-off value (ECOFF), which are valuable in AMR surveillance, cannot be measured using these methods. However, molecular techniques can be used for identification of some bacterial species (e.g. Campylobacter, Enterococcus).

Recommendations regarding choice of susceptibility testing methods: for purposes of surveillance and in the interest of comparability of the data provided by each country in the region, standardized-quantitative methods providing MIC (expressed in µg/ml) are recommended. Antimicrobial susceptibility testing should be conducted using standardized dilution methods (either agar-dilution or broth-dilution methods) as described by the Clinical and Laboratory Standards Institute (CLSI) (CLSI, 2013) or EUCAST. The approved CLSI guideline VET01-A4/Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated From Animals (CSLI, 2013) should be used. Each MIC determination must include quality control bacterial strains as recommended by CLSI to monitor performance and reproducibility of the test system (Table 4.3).

4.3.2 Interpretation of test results

Currently, two different types of interpretive criteria are available: **clinical breakpoints** and **epidemiological cut-off values**. The objective of the work at hand will determine which criteria must be applied.

(1) Clinical breakpoints
Clinical breakpoints (CBPs) are numbers used exclusively to predict the treatment outcome using the standard dosing regimens. The values are beneficial to generate surveillance data that are used to guide prescribers in their selection of empirical treatments and to update treatment guidelines and protocols (CLSI, 2011).

(2) Epidemiological cut-off values
Epidemiological cut-off values (ECV or ECOFFs) are used to describe MIC distributions of bacteria without clinical context. They are determined on the basis of the distribution of MICs for an antimicrobial and a bacterial species and will not be changed by changing circumstances (e.g. sampling time, sources, geographical origins). The population that clearly departs from the normal population (or wild type) is categorized as "non-wild type". ECOFFs are valuable for early detection of decreased susceptibility, but inappropriate to use to determine the percentage of clinical resistance. These cut-off values do not take into account the results of clinical efficacy studies, dosage and route of administration of the antimicrobial agent, or the drug's pharmacokinetic and pharmacodynamic parameters in the animal species concerned (CLSI, 2011). A bacterial species defined as non-wild type may or may not respond clinically to antimicrobial treatment. EUCAST introduced the term "**microbiological resistance**" and presents ECOFFs for antimicrobials against a wide range of bacteria (EFSA, 2012a and EFSA, 2019). Table 4.4 presents a comparison of CBPs and ECOFFs and more detailed information on CBPs and ECOFFs can be found in CLSI (2011).


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Table 4.3 Selected references for some monitoring procedures

Procedures	Test procedure	CLSI reference[a]
General procedures	Disk diffusion susceptibility tests	Section 7, VET01-A4
	Broth and agar dilution susceptibility testing	Section 8, VET01-A4
	Preparation of antimicrobial agents	Section 9, VET01-A4
AST procedures for target organisms	Disk diffusion susceptibility methods	Section 7.2, VET01-A4
	Agar dilution procedure for *E. coli, Salmonella* spp. and *Enterococcus* spp.	Section 10, VET01-A4
	Broth dilution procedure for *E. coli, Salmonella* spp. and *Enterococcus* spp.	Section 11, VET01-A4
	Disk diffusion testing of *Campylobacter jejuni*	Section 7.6, VET01-A4
	Agar dilution testing for *C. jejuni*	Section 10.2, VET01-A4
	Broth dilution for *Campylobacter* spp.	Section 12.3, VET01-A4
Complementary tests	*Enterococcus* spp.	Section 14, VET01-A4
		Section 16.3, VET01-A4
	Extended-spectrum beta lactamase	Table 9I, VET01S
		Section 16.4, VET01-A4
		Table 9A and 9B, VET01S

[a] It is important to note that CLSI standards are updated regularly and references as cited may change over time; although these excerpts were cited for quick referencing, it is important to highlight that it is better to search for the latest CLSI standards.

Source: CLSI (2013) and CLSI (2015)

(3) Interpretive criteria and AMR surveillance purpose

The AST results originating from **AMR monitoring programmes on healthy animals and food of animal origins** are for public health purposes and should generally be interpreted based on ECOFF values. Comparing resistance levels in the isolates from animals and food with those from humans using ECOFFs will facilitate early detection of acquired resistance.

To compare the AST results for the human and animal isolates, **the data** should be interpreted with CBPs. The priority is assigned to CLSI CBPs. For the antimicrobials where CLSI CBPs do not exist, EUCAST CBPs should be used. For the antimicrobials where CLSI and EUCAST CBPs do not yet exist, interpretive criteria from other guidelines (e.g. WHO, 2017) may be applied. These criteria are included in the footnotes to Tables 4.6A, 4.6B and 4.6C.

Note that there is still no standardized and harmonized approach to defining ECOFF and the national AMR surveillance programmes currently using ECOFF do not all use the same values and should therefore be compared with care. There is still a considerable need to harmonize the process for agreeing ECOFF.

In summary, AST data intended for surveillance should be interpreted using ECOFF and those intended for recommending clinical therapy should be interpreted using CBPs. These two cannot be interchanged.

Table 4.4 Comparison of CBPs and ECOFFs

	Clinical breakpoint	Epidemiological cut-off value
Applications	• Predicting treatment outcomes • Selecting empirical treatments • Updating treatment guideline and protocol	• Detecting changes in the intrinsic vulnerability of bacterial populations against specific antimicrobial agents
Relevance	• Clinically relevant	• Epidemiologically relevant
Threshold	• Breakpoint	• Cut-off value
Considerations for determining threshold	• Pharmacokinetic and pharmacodynamic properties of the drug in the species • Clinical efficacy studies • Dosing • Route of administration of AM agents	• MIC distribution data
Categories	• Susceptible • Intermediate • Resistant	• Wild-type • Non-wild-type
Application is specific to animal species of interest	• Yes	• No
Application is affected by changes in breakpoints in light of new information	• Yes	• No

4.3.3 Quality control (QC) in antimicrobial susceptibility testing

The QC for AST aims to ensure that the only variable in the test is the microorganism's properties determining its reaction to the antimicrobial drug. However, AST is understandably vulnerable to other factors that may influence the results such as quality of media and reagents, viability of microorganisms being tested, and the person performing the test. Thus, the goals of a quality control programme for AST are to monitor and ensure consistency of:

- the precision/repeatability and accuracy of the susceptibility test procedure;
- the performance of reagents and the viability of the microorganisms used in the test; and
- the performance of the persons who carry out the tests and interpret the results.

Quality control (QC) guidelines, including the use and maintenance of reference strains, are described in Chapter 15 of CLSI (2013).

Table 4.5 Section references for some test procedures

Test procedure	CLSI reference[a]
Quality control guidelines	Section 15.1, VET01-A4
Disk diffusion	Section 15.4, VET01-A4
Dilution susceptibility tests	Section 15.6, VET01-A4
Reference strains	Section 15.3, VET01-A4 Table 3, VET01S
Acceptable quality control ranges	Tables 4 and 5, VET01S
Troubleshooting guides	Appendix D1, D2, VET01-A4

[a] It is important to note that CLSI standards are updated regularly and references as cited may change over time; although these excerpts were cited for quick referencing, it is important to highlight that it is better to search for the latest CLSI standards.

Source: CLSI (2013) and CLSI (2015)

4.3.4 Harmonized panel of antimicrobials for monitoring

The common test panel of antimicrobial agents in the monitoring programme should be concise but provide valuable information about the possible resistance to a much broader group of agents. It should suggest additional antimicrobial agents to be tested as well. The list of recommended antimicrobial agents to be included in AMR monitoring is given in Table 4.6A, Table 4.6B and Table 4.6C together with interpretive criteria and recommended concentration ranges. Additional antimicrobial agents can be included, depending on country needs and preferences.

Table 4.6A Antimicrobial panel and interpretive criteria for *Salmonella* spp.and *E. coli*

Relevant antimicrobial group[f]	Clinical breakpoint (µg/ml)		ECOFF (EUCAST)		Advised concentration range to be tested (µg/ml)	Classification and prioritization[g]
	CLSI	EUCAST	*Salmonella* spp.	*E. coli*		
Azithromycin	NA	NA	NA	NA	1–64	Highest priority critically important antimicrobials
Cefotaxime[a]	≧4	>2	>0.5	>0.25	0.064–16	
Ceftazidime[a]	≧16	>4	>2	>0.5	0.064–32	
Nalidixic acid	≧32	NA	>16	>16	1–128	
Ciprofloxacin	≧4	>0.5	>0.064	>0.064	0.008–16	
Colistin[c]	NA	>2	>NA[d]	>2	0.125–16	
Gentamicin	≧16	>4	>2	>2	0.25–128	High priority critically important antimicrobials
Streptomycin	NA	NA	>16	>16	1–256	
Meropenem[b]	≧4	>8	>0.125	>0.125	0.008–16	
Ampicillin	≧32	>8	>8	>8	0.5–128	
Chloramphenicol	≧32	>8	>16	>16	1–256	Highly important antimicrobials
Sulphamethoxazole	≧512	NA	NA	>64	1-2048[e]	
Trimethoprim	≧16	>4	>2	>2	0.25-256	
Tetracycline	≧16	NA	>8	>8	1-256	

a, b See Section 4.4.5 for details.
c Colistin is for broth dilution only.
d The EUCAST ECOFF (>2) for colistin was applied for S. Typhiurium and other serotypes, except for S. Enteritidis and S. Dublin where ECOFF >8 was applied according to investigations presented in WHO (2017).
e Can be set at a lower concentration, but experience from the region shows that this is often high.
f Others of particular interest may be added as an option.
g WHO (2016)

Table 4.6B Antimicrobial panel and interpretive criteria for *Campylobacter jejuni* and *C. coli*

Relevant antimicrobial group[b]	Clinical breakpoint (µg/ml)		ECOFF (EUCAST)		Recommended concentration range to be tested (µg/ml)	Classification and prioritization[c]
	CLSI	EUCAST	*C. jejuni*	*C. coli*		
Ciprofloxacin	≧4	>0.5	>0.5	>0.5	0.0625–32	Highest priority critically important antimicrobials
Nalidixic acid	NA[a]	NA	>16	>16	0.0625–32	
Erythromycin	≧32	C. jejuni: >4 C. coli: >8	>4	>8	0.25–128	
Gentamicin	NA	NA	>2	>2	0.125–16	High priority critically important antimicrobials
Streptomycin	NA	NA	>4	>4	0.5–256	
Tetracycline	≧16	>2	>1	>2	0.25–128	Highly important antimicrobials

a ≧32 (Hakanen et al.) as cited by WHO, 2017.
b Clindamycin and others of particular interest may be added as options (See WHO, 2017 for comparison).
c WHO (2016)

Table 4.6A Antimicrobial panel and interpretive criteria for *Enterococcus faecalis* and *E. faecium*

Relevant antimicrobial group[c]	Clinical breakpoint (µg/ml)		ECOFF (EUCAST)		Advised concentration range to be tested (µg/ml)	Classification and prioritization[b]
	CLSI	EUCAST	*E. faecalis*	*E.faecium*		
Erythromycin	≧8	NA	>4	>4	0.25–128	Highest priority critically important antimicrobials
Teicoplanin	≧32	>2	>2	>2	0.125–64	
Vancomycin	≧32	>4	>4	>4	0.5–128	
Ampicillin	≧16	>8	>4	>4	0.25–64	High priority critically important antimicrobials
Gentamicin	NA	>128	>32	>32	1–1 024	
Streptomycin	NA	NA	>512	>128	1–2 048	
Tigecycline	NA	>0.5	NA[a]	NA[a]	0.25–64	
Linezolid	≧8	>4	>4	>4	0.5–64	
Quinusristin/ dalfopristin	≧4	>4	NA	NA	0.25–64	Highly important antimicrobials
Chloramphenicol	≧32	NA	>32	>32	1–128	
Tetracycline	≧16	NA	>4	>4	0.25-128	

a >0.25 is used by EFSA (EFSA, 2012a)
b WHO (2016)
c Other antimicrobials of particular interest may be added as option (See WHO, 2017 for comparison).

Given that this particular surveillance at the human-animal interface is for the purpose of protecting public health, the panel was anchored in the most relevant antimicrobials to humans as per WHO classification (WHO, 2016; WHO, 2017), as well as the recognized general usage data in animals in the region. Additional antimicrobials, particularly those that are specifically relevant to the country, may be added as deemed necessary, noting that the above panel should be included as a priority for the purpose of harmonized monitoring in the region.

As the methodologies referred to above followed the CLSI, reference to clinical breakpoints in accordance with the CLSI will be prioritized.

Recommendation regarding interpretation of AMR tests: it is recommended that countries obtain and store MIC data as these are needed for determining ECOFFs or CBPs as these become available in the future. For now, this current version of the guideline will mainly use the clinical breakpoints from CLSI to interpret the MICs. If clinical breakpoints of some antimicrobial agents are not available in CLSI, the breakpoints from EUCAST will be used. For particular antimicrobial agents where no CBPs are currently available, the MIC frequency distribution shall be reported. The concentration ranges of antimicrobials to be used should cover both the ECOFFs and the CBPs. The region will adapt accordingly as more updated information on these standards becomes available.

4.3.5 Complementary antimicrobial susceptibility testing

Detection of extended-spectrum β-lactamases (ESBL)-producing and carbapenem-resistant bacteria in animal populations is considered very important. Phenotypic testing for ESBL production and carbapenem resistance in Salmonella and *E. coli* should be performed when possible (see Figure 4.5).

It is encouraged to differentiate ESBL, AmpC or ESBL+AmpC phenotypes among extended-spectrum cephalosporin (ESC) resistant Salmonella and *E. coli* isolates. Carbapenemase phenotypes should also be detected. This is to provide better understanding of the epidemiology of AMR and to assess zoonotic risks.

(1) Extended spectrum β-lactamase (ESBL) producing Salmonella and *E. coli*

Cefotaxime and ceftazidime are included in the harmonized panel for routine monitoring. **Salmonella or *E. coli* isolates that are resistant to either cefotaxime or ceftazidime** should be further confirmed for ESBL-production. The phenotypic confirmatory test requires use of ceftazidime, alone and in combination with clavulanate. The ESBL-producing bacteria exhibit cephalosporin/clavulanate synergy. Screening and confirmatory testing for ESBL production can be performed either by standard disk diffusion or standard broth dilution as described in CLSI (2011).

(2) Carbapenemase resistant Enterobacteriaceae

The carbapenemase enzymes belong to several different classes of β-lactamases and no single test yields high sensitivity and specificity for all types of enzymes. Meropenem is considered the optimal compound giving the best compromise between sensitivity and specificity in terms of detecting the majority of carbapenemases (EFSA, 2018). Meropenem should be included in the harmonized panel of antimicrobial agents. Concentration ranges to be tested and interpretive criteria for meropenem are included in Table 4.6A. The isolates resistant to meropenem should be re-tested to confirm such resistance, which is likely to be rare in veterinary and food isolates. It is noted that detected results of carbapenemase-producing Enterobacteriaceae should be reported quantitatively and not as interpreted values, as carbapenemase-producing Enterobacteriaceae often have MIC-values below the CBP. Screening and confirmatory test for CRE can be performed according to CLSI (2012).

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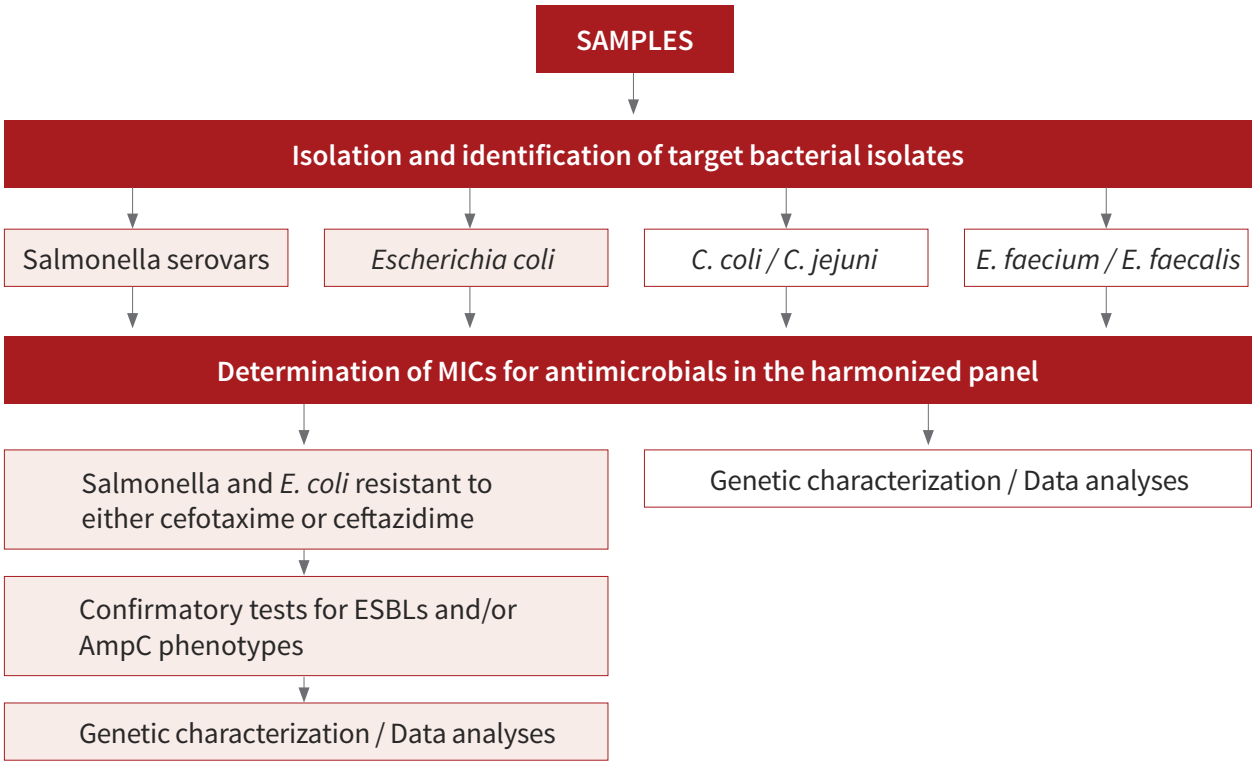
Figure 4.5 Stepwise detection of antimicrobial susceptibility for Salmonella, *E. coli*, Campylobacter and Enterococcus isolates



4.4 Storage of the isolates

The bacterial isolates should be permanently preserved. The methods of storage must ensure viability, safety against loss because of contamination and cross-contamination, absence of changes in the strain properties and absence of phenotypic drift because of genetic instability (EC, 2013). The bacterial isolates can be stored based on ATCC (2015) or NATA (1992). Several methods have been successfully used for the preservation of microorganisms, but among these, cryopreservation and lyophilization are highly utilized for culture collection and industry.

4.4.1 Cryopreservation

Cryopreservation refers to the preservation of biological materials at cryogenic temperatures, generally at -80 °C or -196 °C. Low temperature protects proteins and DNA from denaturation and damage and slows the movement of cellular water. This is appropriate for most non-fastidious bacterial strains. The isolates should be stored at a temperature below -70 °C yielding a medium storage of five years. **Storage at -20 °C and above should be avoided.**

Cryoprotectant agents are essential in cryopreservation. Glycerol and dimethyl sulfoxide (DMSO) are the most common agents, although there are others that have been utilized. It is recommended to use a 20 percent glycerol stock at a final concentration of 10 percent. If the bacterial strain is sensitive to glycerol, a 50 percent DMSO stock can be used at a final concentration of 5 percent. Only reagent-grade DMSO or glycerol should be used. It is important to note that glycerol can be sterilized by autoclaving but DMSO must be sterilized by filtration. Both should be stored in aliquots protected from light. Glycerol is usually prepared in an aqueous solution at double the desired final concentration for freezing. Then it is mixed with an equal amount of cell suspension. For more details, please refer to the ATCC guide ATCC (2015).

4.4.2 Freeze-drying or lyophilization

Lyophilization is the process where water and other solvents are removed from a frozen product via sublimation which occurs when a frozen liquid goes directly to a gaseous state without entering a liquid phase. The freeze-drying process results in a stable, readily rehydrated product. This is often the preferred long-term preservation method in most microbial resource centres because of the low cost of maintenance and ease of transportation of lyophilized cultures.

This process consists of three steps: pre-freezing the product to form a frozen structure; primary drying to remove most water; and secondary drying to remove bound water. For more details, please refer to ATCC (2015) or NATA (1992).

4.4.3 Other methods

In addition to the more commonly used cryopreservation and lyophilization, other methods have been used successfully for the preservation of microorganisms: repeated sub-culturing; preservation on agar beads; overlay of slant-grown cultures; use of silica gel; and other sterile supports.

4.4.4 Additional notes on storage of isolates

Apart from the brief review above of some of the common methods used for storage of isolated, some further observations can be made:

- Most bacterial strains can be freeze-dried, and almost all strains can be cryopreserved and maintained in liquid nitrogen vapour. However, additional care must be taken when preparing fastidious bacterial species for preservation e.g. Campylobacter. The viability of bacterial cells should be checked regularly depending on the storage methods.

- As previously highlighted, the methods of storage must ensure viability, safety against loss because of contamination, absence of changes in the strain properties and absence of phenotypic drift because of genetic instability. The genotypic (and phenotypic) changes relevant to antimicrobial resistance are important to consider when preparing to store isolates, and when interpreting results of susceptibility testing of stored isolates. A comprehensive characterization of cultures on morphological, anatomical physiological, immunological and molecular grounds is a must before and after preservation. Although cultures preserved using lyophilization and cryopreservation showed more genotypic and phenotypic stability, this still needs optimization for accurate results.

- Multiple replicates of each isolate, stored separately, should be prepared. Each isolate to be stored should be properly labelled and documented in the appropriate inventory or database to avoid the risk of losing the traceability of the preserved culture.

- Repeated thawing and freezing will affect the viability and quality of the isolate. Appropriate methods for recovery of preserved culture should be noted, including keeping track how frequently a stored vial has been used.

CHAPTER

5 DATA MANAGEMENT AND REPORTING

5.1 Data recording and storage

Careful consideration should be given to database design in order to store and keep the complex and voluminous information for an undetermined period of time.

5.1.1 Recording and storing antimicrobial resistance data

According to OIE Terrestrial Animal Health Code, Chapter 6.8, Article 6.8.8 (OIE, 2018):

- "The storage of raw (primary, non-interpreted) data is essential to allow the evaluation in response to various kinds of questions including those arising in the future."
- "Consideration should be given to the technical requirements of computer systems when an exchange of data between different systems (comparability or compatibility of automatic recording of laboratory data and transfer of these data between and within resistance surveillance and monitoring programmes) is envisaged. Results should be collected in a suitable national database and recorded quantitatively:

  a. as distributions of MICs in micrograms per millilitre;
  b. or inhibition zone diameters in millimetres."

- "The information to be recorded should include, where possible, the following aspects:

  a. sampling programme;
  b. sampling date;
  c. animal species and production type;
  d. type of sample;
  e. purpose of sampling;
  f. type of antimicrobial susceptibility testing method used;
  g. geographical origin (geographical information system data where available) of herd, flock or animal;
  h. animal factors such as age, condition, health status, identification, sex, breed;
  i. exposure of animals to antimicrobial agents; and
  j. bacterial isolation rate."

- "The reporting of laboratory data should include the following information:

  a. identity of laboratory;
  b. isolation date;
  c. reporting date;
  d. bacterial species, and, where relevant, other typing characteristics, such as serotype or serovar;
  e. phage type, wherever applicable,
  f. antimicrobial susceptibility result or resistance phenotype and
  g. genotype."

5.1.2 Regional template for antimicrobial resistance data collection

As part of its efforts to assist countries in initiating a sustainable AMR surveillance programme in the agriculture sector, FAO RAP, through OSRO/RAS/502/USA, has also developed a regional template as a supplement to the present guideline (See Annex 3 to see template). This is a convenient tool for simple AMR data entry and storage that countries can use to strategically collate AMR data generated and harmonize with the rest of the region. This template has also been prepared in view of the option to transition to more established platforms for AMR data storage and analysis, such as WHONET (See Section 5.3.4 in this guideline).

Although this template presents the opportunity to harmonize formats for ease of communication and possibly future collation of all relevant AMR surveillance data across the region, it is important to note that this is only one of a number of options to the approach that a country may take, and can be modified as needed. It will be important, however, to capture and convey the modifications and changes made using the data definition matrix in Table 5.1.

Table 5.1 Data definition matrix from the regional AMR data collection template

Column title	Definition/description	Data type	Max field size	Options list	Example
Location: The geographical source of isolate					
Identification number	Identification number of the isolate	Text/ Number	12 characters	User defined[a]	H_012_456
Country	Name of country	Text	No limit	User defined[a]	Thailand
State	Name of state (if applicable)	Text	No limit	User defined[a]	-
Province	Name of province (if applicable)	Text	No limit	User defined[a]	Bangkok
City	Name of city (if applicable)	Text	No limit	User defined[a]	Bangkok
District	Name of district (if applicable)	Text	No limit	User defined[a]	Phra Nakhon
Village	Name of village (if applicable)	Text	No limit	User defined[a]	Phra Arthit
Origin: Describes the source of the specimen					
Farm number	Code for the farm (if applicable)	Text/ Number	10 characters	User defineda	F101
Location type	Where the specimen was collected	Coded	3 letters	See LT_List	sla = slaughterhouse
Location	Name/code of location type (if applicable)	Text/ Number	6 characters	User defined[a]	sla-001
Species	Species of the specimen	Coded	3 letters	See SP_List	chi = chicken
Breed	Breed of animal from which sample was taken	Text/ Number	10 characters	User defined[a]	Native
Animal type	Purpose for which the animal was raised	Coded	3 letters	See AT_List	mea = meat producing
Age	Age of the animal source (standardized)	Text/ Number	3 characters	See Age_exp	1 m
Age category	Newborn, young, adult, unknown, other	Coded	3 letters	See AC_List	adu = adult
Market category	Domestic, imported, for export, etc.	Coded	1 letter	See MC_List	d = domestic
Food	Kind of food sampled (if applicable)	Text/ Number	15 characters	User defined[a]	egg

Column title	Definition/description	Data type	Max field size	Options list	Example
Specimen: Description of the specimen from which the isolate was obtained					
Collected by	Name or identifier for specimen collector	Text/ Number	20 characters	User defined[a]	AHW_239
Specimen type	Specimen collected	Coded	15 characters	See WHONET[b]	Cecum
Specimen number	Code for the specimen	Text/ Number	12 characters	User defined[a]	F101_34
Specimen date	Date and time of sampling	Date/ Time	20 characters	User defined[a]	10 Sep 2019
Reason	Purpose for sample collection	Coded	1-3 characters	See RE_List	r = research
Microbiology: Laboratory data of the isolate					
Laboratory	Name/ID of the lab where AST was done	Text/ Number	3 characters	User defined[a]	A12
Local organism code	Isolate number/code from the lab of origin	Text/ Number	50 characters	User defined[a]	A12_W4356
Organism	Bacterial species identification	Coded	3 characters	See WHONET[b]	eco
Serotype	Serotype of the isolate	Coded	No limit	See WHONET[b]	*Escherichia coli* serogrup O11
AST method	Lab method used to obtain AMR data	Text	No limit	User defined[a]	Microbroth dilution
Storage location	Location where the isolate is stored	Text/ Number	10 characters	User defined[a]	Clab
Storage number	Storage number/code of the isolate	Text/ Number	11 characters	User defined[a]	CL_5618

[a] User defined = it is suggested that countries should define naming/coding at the outset.
[b] There is a long list of options in the WHONET platform. If not using WHONET, then should be user-defined.

This regional AMR data collection template (see Annex 3) also contains the list of antibiotics based on the harmonized panel of antimicrobials as listed in Section 4.3.4 of this guideline for all target bacteria (*Escherichia coli, Salmonella* spp., *Enterococcus* spp., *Campylobacter* spp.).

Entries should be made on the respective antibiotic columns where data are available. For antibiotics tested but not listed, additional columns may be added – for ease of future use, these should be placed at the end of the existing columns listed.

Table 5.2 lists the antibiotics included in the regional AMR data collection template.

Table 5.2 List of antibiotics included in the regional AMR data collection template

Code	Antibiotic name	Code	Antibiotic name	Code	Antibiotic name	Code	Antibiotic name
AMP	Ampicillin	CIP	Ciprofloxacin	MEM	Meropenem	TEC	Teicoplanin
AZM	Azithromycin	COL	Colistin	QDA	Quinupristin/ Dalfopristin	TCY	Tetracycline
CTX	Cefotaxime	ERY	Eryhromycin	NAL	Nalidixic acid	TGC	Tigecycline
CAZ	Ceftazidime	GEN	Gentamicin	STR	Streptomycin	TMP	Trimethoprim
CHL	Chloramphenicol	LNZ	Linezolid	SMX	Sulfamethoxazole	VAN	Vancomycin

5.2 Interpretation of results

This may include any of the following: discussion of trends, emerging resistance, discussion of encountered difficulties and inherent biases, relevance of findings, comparison of the situation along the food chain. The following were covered in the OIE Code:

- The number of isolates regarded as resistant should be reported as a proportion of the number of isolates tested, including the defined interpretive criteria used.
- In the clinical setting, breakpoints are used to categorize bacterial strains as susceptible, intermediate or resistant. These clinical breakpoints may be elaborated on a national basis and may vary between member countries.
- For surveillance and monitoring purposes, use of the microbiological breakpoint (also referred to as epidemiological cut-off point) is preferred. This is based on the distribution of MICs or inhibition zone diameters of the specific bacterial species tested.
- When using microbiological breakpoints, only the bacterial population with acquired resistance that clearly deviates from the distribution of the normal susceptible population will be designated as resistant.
- Ideally, data should be collected at the individual isolate level. This will allow antimicrobial resistance patterns to be recorded over time, along with, when available, relevant data on usage of antimicrobial agents and management practices.

5.3 Data management and data analysis

Data should be in electronic format and stored in a structured data management system (example: excel file, LIMS, or any available data management software, such as WHONET) for ease of sharing and further data analysis. The data should be collected and analyzed at local, subnational, and national level and presented in a consistent format grouped by target bacteria as follows:

- All *Salmonella enterica*
- *S. Enteritidis*
- *S. Typhimurium*
- *Serovars* other than *Salmonella Enteritidis* or Typhimurium
- *Escherichia coli*
- All *Campylobacter* spp.
- *C. coli*
- *C. jejuni*
- All *Enterococcus* spp.
- *E. faecalis*
- *E. faecium*

The following are also recommended in the region:

- interpretive criteria used for Minimum inhibitory concentration (MIC) determination if deviating from the recommended susceptibility testing methods;
- description of quality assurance systems;
- results of AST (MIC value);
- results of AST will be in table form for every animal population, broken down by bacterial species;
- qualitative tables to report the result for each antimicrobial tested including:
  - number of isolates tested
  - number of resistant isolates
  - number of fully-susceptible and number of isolates resistant to 1, 2, 3, 4, 5 or >5 antimicrobials of different classes;
- prevalence measures for each organism and antimicrobial combination, preferably as a time series if there is enough data, including confidence intervals;
- quantitative tables to report MIC distributions for each animal in each bacterial species;
- MIC50 and MIC90 for each antibiotic should be calculated;
- prevalence of resistant isolates in the target population and reported for the appropriate epidemiological unit, i.e. animals, flocks, food samples; and
- confidence intervals of the prevalence values expressing the precision of the esimates.

See Tables 5.3, 5.4, 5.5 and 5.6 for some examples.

Table 5.3 Example table for MIC frequency distribution by animal source

Antimicrobial	Isolate sources (No. of isolates)	MIC_{50}	MIC_{90}	% R	Distribution (%) of MICs (µg/ml)													
					0.12	0.25	0.5	1	2	4	8	16	32	64	128	256	512	1024	2048
Azithromycin Azithromycin	Broiler Poultry (n)																		
	Swine (n)																		
	Cattle (n)																		
Cefotaxime	Poultry (n)																		
	Swine (n)																		
	Cattle (n)																		
Ceftazidime	Poultry (n)																		
	Swine (n)																		
	Cattle (n)																		
Nalidixic acid	Poultry (n)																		
	Swine (n)																		
	Cattle (n)																		
Ciprofloxacin	Poultry (n)																		
	Swine (n)																		
	Cattle (n)																		

Antimicrobial	Isolate sources (No. of isolates)	MIC_{50}	MIC_{90}	% R	Distribution (%) of MICs (µg/ml)														
					0.12	0.25	0.5	1	2	4	8	16	32	64	128	256	512	1024	2048
Colistin	Poultry (n)																		
	Swine (n)																		
	Cattle (n)																		
Gentamicin	Poultry (n)																		
	Swine (n)																		
	Cattle (n)																		
Streptomycin	Poultry (n)																		
	Swine (n)																		
	Cattle (n)																		
Meropenem	Poultry (n)																		
	Swine (n)																		
	Cattle (n)																		
Ampicillin	Poultry (n)																		
	Swine (n)																		
	Cattle (n)																		
Chloramphenicol	Poultry (n)																		
	Swine (n)																		
	Cattle (n)																		
Sulphamethoxazole	Poultry (n)																		
	Swine (n)																		
	Cattle (n)																		
Trimethoprim	Poultry (n)																		
	Swine (n)																		
	Cattle (n)																		
Tetracycline	Poultry (n)																		
	Swine (n)																		
	Cattle (n)																		
	TOTAL (N)																		

Table 5.4 Example table showing resistance (%) by bacterial species tested

Antimicrobial	*Campylobacter Species*		
	C. jejuni (n=)	*C. coli (n=)*	Other *Campylobacter* spp. (n=)
Azithromycin	%	%	%
Cefotaxime	%	%	%
Ceftazidime	%	%	%
Nalidixic acid	%	%	%
Ciprofloxacin	%	%	%
Colistin	%	%	%
Gentamicin	%	%	%
Streptomycin	%	%	%
Meropenem	%	%	%
Ampicillin	%	%	%
Chloramphenicol	%	%	%
Sulphamethoxazole	%	%	%
Trimethoprim	%	%	%
Tetracycline	%	%	%

Table 5.5 Example table showing resistance (%) by animal species tested in a reporting period

Antimicrobial	*E. coli* isolates from caecal samples		
	Broilers (n=)	Pigs (n=)	Cattle (n=)
Azithromycin	%	%	%
Cefotaxime	%	%	%
Ceftazidime	%	%	%
Nalidixic acid	%	%	%
Ciprofloxacin	%	%	%
Colistin	%	%	%
Gentamicin	%	%	%
Streptomycin	%	%	%
Meropenem	%	%	%
Ampicillin	%	%	%
Chloramphenicol	%	%	%
Sulphamethoxazole	%	%	%
Trimethoprim	%	%	%
Tetracycline	%	%	%

Table 5.6 Example table showing antimicrobial resistance in Salmonella by animal source, years

Year	Source	2017	2018	2019	2020	2021	2022	2023	2024	2025	2026
Number of isolates tasted	Poultry Swine Cattle	(n) (n) (n)	(n) (n) (n)	(n) (n) (n)	(n) (n) (n)	(n) (n) (n)	(n) (n) (n)	(n) (n) (n)	(n) (n) (n)	(n) (n) (n)	(n) (n) (n)
Antimicrobial	**Isolate source**										
Azithromycin	Poultry	(%), (n)	(%), (n)	(%), (n)	(%), (n)	(%), (n)	(%), (n)	(%), (n)	(%), (n)	(%), (n)	(%), (n)
	Swine	(%), (n)	(%), (n)	(%), (n)	(%), (n)	(%), (n)	(%), (n)	(%), (n)	(%), (n)	(%), (n)	(%), (n)
	Cattle	(%), (n)	(%), (n)	(%), (n)	(%), (n)	(%), (n)	(%), (n)	(%), (n)	(%), (n)	(%), (n)	(%), (n)
Cefotaxime	Poultry	(%), (n)	(%), (n)	(%), (n)	(%), (n)	(%), (n)	(%), (n)	(%), (n)	(%), (n)	(%), (n)	(%), (n)
	Swine	(%), (n)	(%), (n)	(%), (n)	(%), (n)	(%), (n)	(%), (n)	(%), (n)	(%), (n)	(%), (n)	(%), (n)
	Cattle	(%), (n)	(%), (n)	(%), (n)	(%), (n)	(%), (n)	(%), (n)	(%), (n)	(%), (n)	(%), (n)	(%), (n)
Ceftazidime	Poultry	(%), (n)	(%), (n)	(%), (n)	(%), (n)	(%), (n)	(%), (n)	(%), (n)	(%), (n)	(%), (n)	(%), (n)
	Swine	(%), (n)	(%), (n)	(%), (n)	(%), (n)	(%), (n)	(%), (n)	(%), (n)	(%), (n)	(%), (n)	(%), (n)
	Cattle	(%), (n)	(%), (n)	(%), (n)	(%), (n)	(%), (n)	(%), (n)	(%), (n)	(%), (n)	(%), (n)	(%), (n)
Nalidixic acid	Poultry	(%), (n)	(%), (n)	(%), (n)	(%), (n)	(%), (n)	(%), (n)	(%), (n)	(%), (n)	(%), (n)	(%), (n)
	Swine	(%), (n)	(%), (n)	(%), (n)	(%), (n)	(%), (n)	(%), (n)	(%), (n)	(%), (n)	(%), (n)	(%), (n)
	Cattle	(%), (n)	(%), (n)	(%), (n)	(%), (n)	(%), (n)	(%), (n)	(%), (n)	(%), (n)	(%), (n)	(%), (n)
Ciprofloxacin	Poultry	(%), (n)	(%), (n)	(%), (n)	(%), (n)	(%), (n)	(%), (n)	(%), (n)	(%), (n)	(%), (n)	(%), (n)
	Swine	(%), (n)	(%), (n)	(%), (n)	(%), (n)	(%), (n)	(%), (n)	(%), (n)	(%), (n)	(%), (n)	(%), (n)
	Cattle	(%), (n)	(%), (n)	(%), (n)	(%), (n)	(%), (n)	(%), (n)	(%), (n)	(%), (n)	(%), (n)	(%), (n)
Colistin	Poultry	(%), (n)	(%), (n)	(%), (n)	(%), (n)	(%), (n)	(%), (n)	(%), (n)	(%), (n)	(%), (n)	(%), (n)
	Swine	(%), (n)	(%), (n)	(%), (n)	(%), (n)	(%), (n)	(%), (n)	(%), (n)	(%), (n)	(%), (n)	(%), (n)
	Cattle	(%), (n)	(%), (n)	(%), (n)	(%), (n)	(%), (n)	(%), (n)	(%), (n)	(%), (n)	(%), (n)	(%), (n)
Gentamicin	Poultry	(%), (n)	(%), (n)	(%), (n)	(%), (n)	(%), (n)	(%), (n)	(%), (n)	(%), (n)	(%), (n)	(%), (n)
	Swine	(%), (n)	(%), (n)	(%), (n)	(%), (n)	(%), (n)	(%), (n)	(%), (n)	(%), (n)	(%), (n)	(%), (n)
	Cattle	(%), (n)	(%), (n)	(%), (n)	(%), (n)	(%), (n)	(%), (n)	(%), (n)	(%), (n)	(%), (n)	(%), (n)
Streptomycin	Poultry	(%), (n)	(%), (n)	(%), (n)	(%), (n)	(%), (n)	(%), (n)	(%), (n)	(%), (n)	(%), (n)	(%), (n)
	Swine	(%), (n)	(%), (n)	(%), (n)	(%), (n)	(%), (n)	(%), (n)	(%), (n)	(%), (n)	(%), (n)	(%), (n)
	Cattle	(%), (n)	(%), (n)	(%), (n)	(%), (n)	(%), (n)	(%), (n)	(%), (n)	(%), (n)	(%), (n)	(%), (n)
Meropenem	Poultry	(%), (n)	(%), (n)	(%), (n)	(%), (n)	(%), (n)	(%), (n)	(%), (n)	(%), (n)	(%), (n)	(%), (n)
	Swine	(%), (n)	(%), (n)	(%), (n)	(%), (n)	(%), (n)	(%), (n)	(%), (n)	(%), (n)	(%), (n)	(%), (n)
	Cattle	(%), (n)	(%), (n)	(%), (n)	(%), (n)	(%), (n)	(%), (n)	(%), (n)	(%), (n)	(%), (n)	(%), (n)

5.3.1 Minimum inhibitory concentration frequency distributions

For the purpose of continuous monitoring, data should not be reported only as the number and percentage of susceptible or resistant isolates. It should be reported as Minimum inhibitory concentrations (MICs) to allow comparisons, even if the criteria change overtime. This allows for subsequent analysis, especially when different interpretive criteria are used or the criteria change. MIC50 and MIC90 values, which are respectively the MIC value at which ≥50 percent and ≥90 percent of the isolates in a test population are inhibited, as well as the range of values obtained are important parameters for reporting results of susceptibility testing when multiple isolates of a given species are tested. This should always be presented as concentrations on the standard AST dilution series.

5.3.2 Report on the prevalence of resistance/susceptibility in specific subpopulation of bacteria of interest

For Salmonella, it is recommended that the number of serovars should be reported. Resistance to some antimicrobial agents can be associated with particular Salmonella serovars. MIC distributions for *S. Typhimurium* and *S. Enteritidis* should be reported separately because of their public health significance. *S. Derby* may be also reported separately for pigs. The other serovars may be grouped together and reported for each study population separately. *C. jejuni* and *C. coli* show marked differences in the prevalence of resistance to different antimicrobial agents. Both species should be reported separately.

5.3.3 Report of interpreted isolate data

Reports should include information on the number of fully susceptible isolates and the number of isolates resistant to one, two, three, four, five and more than five antimicrobial agents tested. This will facilitate reporting of multiple resistance. It is important that results for additional antimicrobial agents are not included to ensure a fair comparison of resistance data and the number of multidrug-resistant isolates among countries. An example is shown in Table 5.7, but countries are also encouraged to provide visual information using figures and infographics for ease of communicating complex data.

Table 5.7 Multidrug-resistant Salmonella by animal source

Isolate Source	Poultry	Swine	Cattle
Number of isolates tasted	**(n)**	**(n)**	**(n)**
Resistance pattern			
No resistance detected	(%), (n)	(%), (n)	(%), (n)
Resistance ≥ 1 CLSI class	(%), (n)	(%), (n)	(%), (n)
Resistance ≥ 2 CLSI classes	(%), (n)	(%), (n)	(%), (n)
Resistance ≥ 3 CLSI classes	(%), (n)	(%), (n)	(%), (n)
Resistance ≥ 4 CLSI classes	(%), (n)	(%), (n)	(%), (n)
Resistance ≥ 5 CLSI classes	(%), (n)	(%), (n)	(%), (n)

5.3.4 Epidemiological analyses of various antibiogram patterns using WHONET

The epidemiological studies reveal several facets of movement of resistant isolates between animals and between humans and animals that facilitate understanding of the genesis and spread of antimicrobial resistance. This can be done manually but efficient and real-time analyses demands the use of software. The World Health Organization (WHO) has developed an easy-to-use and freely downloadable software called WHONET, which is specifically for drug resistance data. It is primarily used in the human sector but has provision to analyze data from food, the environment and animals. It has the capability to analyze data generated by the disk diffusion, MIC and e-test technologies. More details are available at: (http://www.whonet.org/index.html).

The principal goals of this software are: (i) to enhance local use of laboratory data; and (ii) to promote national and international collaboration through the exchange of data. WHONET can be used by individual laboratories or as a part of a national and international surveillance network. At present the software is available in 17 languages and used in over 80 countries around the world managing data from over 1 000 clinical, public health, veterinary and food laboratories.

WHONET analytical tools facilitate understanding of the local epidemiology of microbial populations, selection of antimicrobial agents, identification of hospital and community outbreaks, and recognition of quality assurance problems in laboratory testing. At present WHONET can handle results from the testing of bacteria, fungi, and parasites, and virology is currently a priority area for further development.

This freely downloadable software has a user-friendly interface permitting many types of analysis including isolate line-listings and summaries such as organism frequencies over time, antimicrobial susceptibility test statistics, zone diameter and MIC histograms, antibiotic scatterplots and regression curves, and antibiotic resistance profile line listings and summaries.

Existing AMR data files (e.g. excel files) can also be exported into WHONET using BacLink. Please note that the template provided in Annex 3 aligns with the data fields and configuration for WHONET for ease of use and collation of AMR data from multiple laboratories.

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GLOSSARY

Active surveillance	"Active surveillance describes an activity that is designed and initiated by the prime users of the data." (FAO, 2014, p. 1).
Agar dilution antimicrobial susceptibility test	"An *in vitro* antimicrobial susceptibility test method conducted using serial concentration of an antimicrobial agent incorporated into an agar growth medium in separate Petri dishes that are inoculated with one or more properly spaced, standardized bacterial suspensions to determine the minimum inhibitory concentration." (CLSI, 2011, p. 3).
Agar disk diffusion antimicrobial susceptibility test	"An *in vitro* antimicrobial susceptibility test method conducted using disks impregnated with a specified single concentration of an antimicrobial agent applied to the surface of a known and specific agar medium that has been inoculated with the test organism." (CLSI, 2011, p. 3). NOTE: The diameter of the zone of growth inhibition that results from the diffusion of an antimicrobial agent from the disks is measured with calipers or a ruler and recorded in millimetres. Zone diameters are recorded and interpreted according to CLSI or EUCAST standards.
Antimicrobial agent	"Any substance of natural, semi-synthetic, or synthetic origin that at *in vivo* concentrations kills or inhibits the growth of microorganisms by interacting with a specific target." (FAO and WHO, 2015, p. 4).
Antimicrobial resistance (AMR)	"The ability of a microorganism to multiply or persist in the presence of an increased level of an antimicrobial agent relative to the susceptible counterpart of the same species." (FAO and WHO, 2015, p. 5).
AMR surveillance	"The continuous, intensive, targeted, and nonrandom collection of data on the incidence, prevalence, and spread or antimicrobial resistant bacteria and antimicrobial resistant genes." (CLSI, 2011, p. 5).
AMR monitoring	"The continuous routine measurement and analysis of antimicrobial susceptibility testing information to detect trends." (CLSI, 2011, p. 5).
Broth dilution antimicrobial susceptibility test	"An *in vitro* antimicrobial susceptibility test conducted using a serial concentration of an antimicrobial agent incorporated in liquid nutrient media that are inoculated with a standardized bacterial suspension to determine the minimal inhibitory concentration of an antimicrobial agent. When this procedure is carried out in test tubes, it is referred to as broth macrodilution; when performed in microdilution plates, it is called broth microdilution." (CLSI, 2011, p. 3)

Clinical interpretive criteria (Clinical breakpoint)	"Minimal inhibitory concentrations (MICs) or zone diameter value used to indicate "susceptible", "intermediate" or "resistant" categories." (CLSI, 2013, p. 3). "**Susceptible** – a category that implies that an infection resulting from the strain may be appropriately treated with the dosage regimen of an antimicrobial agent recommended for that type of infection and infecting species, unless otherwise indicated." (CLSI, 2013, p. 3). "**Intermediate** – a category that implies that an infection resulting from the isolate may be appropriately treated in body sites where the drugs are physiologically concentrated or when a high dosage of drug can be used; also indicates a "buffer zone" that should prevent small, uncontrolled, technical factors from causing major discrepancies in the interpretations." (CLSI, 2013, p. 3). "**Resistant** – resistant strains are not inhibited by the usually achievable concentrations of the antimicrobial agent with normal dosage schedules and/or gall in the range where specific microbial resistance mechanisms are likely, and clinical outcome has not been predictable in effectiveness studies." (CLSI, 2013, p. 4).
Commensal bacteria	Microorganisms participating in a symbiotic relationship in which one species derives some benefit whereas the other is unaffected. Generally commensal microorganisms are considered to be non-pathogenic in their normal habitat but may, in certain circumstances, become opportunistic pathogens. (FAO and WHO, 2015.)
Domestically produced animals	Animals born, hatched, or bred within the country of slaughter, or animals that spent part of their breeding life in the slaughter country (EFSA, 2014).
Epidemiological cut-off value (ECV)	Separates a population into isolates with and those without acquired or mutational resistance based on their phenotypic MIC value. ECVs are normally established on the basis of the MIC distribution data (phenotype) created from testing isolates derived from geographically diverse laboratory surveys. (CLSI, 2013 and CLSI, 2016). "**Non-wild type** – describes isolates with minimal inhibitory concentrations above the epidemiologic cutoff value. "(CLSI, 2011, p. 5). "**Wild type** – describes isolates with minimal inhibitory concentrations below the epidemiological cutoff value." (CLSI, 2011, p. 5).
Food-borne pathogen	A pathogen that typically causes human disease(s) or illness through consumption of food contaminated with the pathogen and/or the biological products produced by the pathogen. (FAO and WHO, 2015.)
Food producing animals	Animals raised for the purpose of producing food for humans. (CLSI, 2011).

Harmonized monitoring of AMR	AMR monitoring activities aimed at providing comparable information on the occurrence of antimicrobial resistance in zoonotic and commensal bacteria. (EFSA, 2014).
Livestock	Livestock means any domestic or domesticated animal including bovine (including buffalo and bison), ovine, porcine, caprine, equine, poultry and bees raised for food or in the production of food. The products of hunting or fishing of wild animals shall not be considered part of this definition. (Codex Alimentarius Commission and FAO, 2007).
Intermediately susceptible bacteria	Bacterial isolates that demonstrate MICs similar to concentrations attained in blood or tissues. The intermediate category implies clinical efficacy only in body sites where the drugs are physiologically concentrated or when a higher than-normal dosage of a drug can be used safely.
Minimal inhibitory concentration (MIC)	"The lowest concentration of an antimicrobial agent that inhibits visible growth of a bacterium in an agar or broth dilution susceptibility test." (CLSI, 2011, p. 3).
MIC50	"The lowest minimal inhibitory concentration at which at least 50 percent of the isolates in a test population are inhibited." (CLSI, 2011, p. 4).
MIC90	"The lowest minimal inhibitory concentration at which at least 90 percent of the isolates in a test population are inhibited." (CSLI, 2011, p. 4).
One Health approach	An integrated approach for preventing and mitigating health threats at the animal-human-plant-environment interfaces with the objective of achieving public health, food and nutrition security, sustainable ecosystems and fair trade facilitation.
Passive surveillance	"Passive surveillance describes surveillance systems where information on disease events is brought to the attention of the veterinary authorities without them actively seeking it." (FAO, 2014, p. 183).
Resistant bacteria	Bacterial isolates whose growth is not inhibited by the usually achievable concentration of the agents with normal dosage schedules and/or fall in the range where specific microbial resistant mechanism are likely and clinical outcome has not been predictable in effectiveness studies. (CLSI, 2013)
Susceptible bacteria	Bacterial isolates or strains causing infections. These may be appropriately treated with the dosage regimen of an antimicrobial agent recommended for that type of infection and infecting species, unless otherwise indicated.

ANNEXES

Annex 1

Sample size calculation for estimating resistance levels in commensal bacteria

This annex presents details and examples for calculating the size of a sample for estimating levels in commensal bacteria that was referred to earlier in Section 2.6.1.

Step 1: Calculate the sample size of commensal bacteria needed. The number of bacterial isolates to be subjected to susceptibility testing should be large enough to estimate reliably the prevalence of resistance. Sample size will depend on the initial or expected prevalence of resistance, the desired level of statistical confidence and the desired power to detect a difference over time.

The objective of monitoring and surveillance in commensal bacteria is to allow calculation of the proportion of resistance to a particular antimicrobial drug in the relevant livestock sectors in the country, and the detection of changes in this proportion over time. The sample size estimates in a large population are shown in Table A1.1. If the expected prevalence of the resistance gene or phenotype of interest is not known or if all possible prevalences should be covered, it is recommended to use 50 percent prevalence as a reference as this requires the largest sample size, i.e. for 95 percent confidence level and 5 percent precision, 384 isolates should be subject to AST. Note that a sample size calculation needs to be conducted for each relevant livestock sector or food type.

The formula for calculating the sample size required to estimate the prevalence of a specific resistance gene or phenotype within a targeted bacteria species is:

Formula 1: $N = [Z^2 \times (P) \times (1-P)]/e^2$

Where N = Total bacterial isolates to be tested per year, P = Prevalence of the resistance gene or phenotype, Z = The standard normal deviation, typically set at 95 percent confidence level (z=1.96) and e = Error (typically 5 percent or 0.05). To facilitate the calculation, online calculation tools are available, e.g. https://select-statistics.co.uk/calculators/sample-size-calculator-population-proportion/

Example for sample size calculation in commensal bacteria

If the expected prevalence of target bacteria is not known, it is recommended to assume it is 50 percent because this will need the largest sample size. An error of 5 percent and a confidence level of 95 percent are set for sample size estimation. Therefore, the sample size estimate N is calculated as follows:

$= [1.96^2 \times 0.50 \times (1-0.50)]/0.05^2$

$= 384.16$

= 384 bacterial isolates/year

Table A1.1 Sample size estimates for prevalence in a large population

	90% Level of confidence			95% Level of confidence		
Expected prevalence[a]	Desired precision			Desired precision		
	10%	5%	1%	10%	5%	1%
10%	24	97	2 429	35	138	3.445
20%	43	173	4 310	61	246	6.109
30%	57	227	5 650	81	323	8.003
40%	65	260	6 451	92	369	9.135
50%	68	270	6 718	96	384	9.512
60%	65	260	6 451	92	369	9.135
70%	57	227	5 650	81	323	8.003
80%	43	173	4 310	61	246	6.109
90%	24	97	2 429	35	138	3.445

[a] Fifty percent is used when the expected prevalence is not known, or when the intention is to test multiple antibiotics each of which would have a different prevalence.

Source: OIE (2017)

Step 2: Calculate the number of specimens (e.g. caeca, meat samples) to be collected, from which the desired number of isolates will be obtained. This will depend on the frequency of isolation of the bacteria targeted. In most cases, commensal bacteria are present in all animals (Table A.1.2), thus the number of specimens to be collected will be equal to the estimated sample size of bacterial isolates needed. In some occasions where known recovery is less than 100 percent, more samples should be taken to ensure that the target number of isolates is achieved. If the recovery is near 100 percent, the number of specimens is the same or slightly higher than the number of isolates needed.

Step 3: Factor in "missingness". To account for potential missing data or loss of specimens for logistical reasons, a 5 percent "missingness" factor should be taken into account. Therefore, the number of epidemiological units to be sampled yearly should be inflated by 5 percent (EFSA, 2014). Loss because of storage (2 percent) may also be added in.

Table A1.2 Example calculation of the number of specimens needed for estimating resistance level in commensal bacteria

Species	Expected prevalence of the targeted bacteria in the animal population[a] (Hypothetical examples only)	Sample size (n= 384 / x, where x is expected prevalence rate of target bacteria	"Missingness" factored in (+ 5 percent of actual sample size)
E. coli	*100%*	384	404
E. faecium and *E. faecalis*	*80%*	480	504

[a] These values are just examples to demonstrate calculations; actual values should be obtained from findings from local studies, or if they cannot be determined, from other countries in the region with very similar settings.

To ensure a biologically meaningful, statistically-based sampling strategy that reflects representativeness, considerations for sampling listed in Section 2.7 should be taken. This includes: Section 2.7.1 Health status of the animal sources; Section 2.7.2 Emphasis on domestic production; Section 2.7.3 Sampling by probability proportional to size; Section 2.7.4 Sampling frequency; Section 2.7.5 Ensuring isolate representativeness/uniqueness and avoiding sample duplication; Section 2.7.6 Streamlining of respective mandates; and Section 2.7.7 Non-random sources in monitoring and surveillance of AMR. If several specimens are collected from the same farm, it is recommended to pool the samples for further microbiological analysis.

Annex 2

Sample size calculation for surveillance of food-borne zoonotic bacteria

This annex presents details and examples for calculating the sample size of food-borne zoonotic bacteria when carrying out surveillance that was referred to earlier in Section 2.6.2.

Step 1: The number of isolates can be calculated using Formula 1 in Annex 1. However, as the prevalence of resistance cannot be expected to be 100 percent, adjustments are required. For example, 21.6 percent of *Campylobacter coli* sampled in slaughter pigs in European countries was reported to be resistant to erythromycin (EFSA, 2018). Using **Table A1.1**, at least 246 isolates would be required to estimate the prevalence of resistance (95 percent confidence and 5 percent error).

If the expected frequency of resistance is uncertain, assuming a prevalence of 50 percent provides the most conservative number, i.e. for a 95 percent confidence level and 5 percent error, 384 isolates should be subject to AST. Note that a sample size calculation needs to be conducted for each relevant livestock sector or food type and for each bacteria species. Formula 1 shown in Annex 1 is applicable and **Table A2.1** can be consulted.

Step 2: In order to obtain the targeted number of isolates, the necessary number of samples needs to be calculated, taking into account the expected prevalence of the zoonotic pathogen. Examples for this adjustment are shown in Table A.2.1 using hypothetical examples. If samples are collected to test for several food-borne zoonotic bacteria, the expected prevalence of the bacteria with the lowest prevalence should be used as it will yield the highest sample size.

Table A2.1 Example calculation for adjusting the sample size for food-borne zoonotic bacteria at a specified prevalence <100 percent

Species	Expected prevalence of zoonotic isolates[a] (Hypothetical examples only)	Sample size (n= 246 / x, where x is expected farm-level prevalence)	"Missingness" factored in (+ 5 percent of actual sample size)
Salmonella spp.	*40%*	384/0.40 = 960	1 008 (pooled samples)[b]
Campylobacter spp.	*10%*	384/0.10 = 3 840	4 032 (pooled samples)[b]

[a] These values are just examples to demonstrate calculations; actual values should be obtained from findings from local studies, or if they cannot be determined, from other countries in the region with very similar settings.

[b] Unlike the commensal indicator bacteria, food-borne pathogens are less common and are not present in all animals in an affected farm. The lower the expected prevalence of the pathogen, the higher the number of samples needed to obtain one isolate. Pooling generally increases sensitivity. If all animals on an affected farm have the bacteria, then the pool size is 1. But if the prevalence is lower, the pool size needs adjusting. In poultry, the pool size commonly used for caecum is 10 per batch based on Campylobacter, which is the hardest to isolate. The pool size for boot swabs in farms is five pairs per flock, equivalent to 300 grams of faecal samples. Note that if samples are not pooled, the number of samples has to be increased according to the within-herd prevalence.

Example for AMR monitoring in Salmonella in farm animals year 1

Target population and sampling frame

The complete list of farms (sampling frame) with the type and number of animals should be provided. The target population is animals at farm, flocks of broilers and laying hens in production, which is recorded for a one-year period starting from 1 January to 31 December. The sampling frame should be updated regularly.

Sample size estimate for estimating Salmonella resistance in the first year

If the prevalence of resistance among Salmonella isolates in farm animals is unknown, the estimated prevalence at 50 percent can be used for sample size calculation for isolates. In this example, the prevalence of resistance against relevant antibiotics among Salmonella is assumed to be 50 percent because this is the most conservative and covers all possible prevalence options.

The required number of isolates for AST = 384 Salmonella isolates/year (see Table A2.1)

The number of samples should then be calculated based on the prevalence of *Salmonella* spp. The samples should be pooled samples from different farms, consisting of at least ten different locations per farm. If the prevalence of Salmonella occurrence, for example, is 40 percent of all targeted farms, then the required sample size is calculated as follows (100/40=2.5):

The required number of specimens = 384x2.5 = 960 samples

Loss because of transportation (5 percent) = 960×1.05 = 1 008 samples

Loss because of storage (2 percent) = 1 008×1.02 = 1 028 samples

Total number of target specimens = 1 028 samples/year

The estimated total number is **at least 1 028 samples/year.**

The samples collected should be approximately equally distributed over the year to cover different seasons. It is suggested that sampling should be performed two or three times per year. For example, if the sampling is to take place three times per year it can be done as follows: period 1, January to April; period 2, May to August; and period 3, September to December.

If sampling from carcasses, the prevalence of pathogen presence on a randomly selected carcass needs to be taken into account. The lower this value, the higher the sample size will be. The impact of this on the total sample size is likely to be considerable. With the progressive success of national control programmes and/or initiatives on food safety, the animal level prevalence of food-borne pathogens will continue to decline in the years to come.

For slaughterhouses, the total sample size should be divided between them according to the relative throughput of the establishments. If a country pursues this approach, the same specimens may be used for the isolation of commensal bacteria (see Section 2.6), since the sample size for food-borne pathogens will be larger than the required smaller sample size for commensal bacteria.

In subsequent years, the number of samples depends on the extent to which differences in resistance prevalence should be detected. The smaller the differences that the surveillance should be able to detect, the larger the required sample size. For an increase of 10 percent from 40 percent to 50 percent to be detected, approximately the same sample size will be required as for establishing the prevalence in year 1. However, if a 5 percent increase should be detected, the number of isolates to be subject to AST will increase substantially from 384 to >1 500.

Formula 2 (Dohoo, Martin and Stryne, 2009) is applicable:

Formula 2:

$$N = \frac{((Z_{alpha} \times \sqrt{(2\ pq}) - (Z_{beta} \times \sqrt{(p_1\ q_1 + p_2\ q_2}))2}{(p_1 - p_2)^2}$$

Where N = Total bacterial isolates to be subject to AST per year, p=(p1+p2)/2, p1 = prevalence year 1, p2 = prevalence year 2, q=1-p, q1 = 1-p1, q2=1-q2, Zalpha = typically set at 95 percent confidence level (z=1.96), Zbeta = typically set at 80 percent confidence level (z=-0.84).

To facilitate the calculation, online calculation tools are available, e.g. https://select-statistics.co.uk/calculators/sample-size-calculator-two-proportions/

Annex 3

Regional template for the surveillance of antimicrobial resistance in agriculture

Download regional template here

Part 1: Data inputs for sample location and origin details

Identification number	COUNTRY	State	Province	City	District	Village

Farm number	Location type[1]	Location	Species[2]	Breed	Animal type[3]	Age[4]	Age category[5]	Market category[6]	Food

[1] See Part 1A for list of options for location type
[2] See Part 1B for list of options for species
[3] See Part 1C for list of options for animal type
[4] See Part 1D for explanatory note on encoding age
[5] See Part 1E for list of options for age category
[6] See Part 1F for list of options for market category

Part 2: Data inputs for specimen and microbiology details

Collected by	Specimen type	Specimen number	Specimen date	Reason[7]	Laboratory	Local organism code	Organism	Serotype	AST Method	Storage location	Storage number

[1] See Part 2A for list of options for reasons for sample collection

Part 3: Data inputs for antibioticc list

AMP	AZM	CTX	CAZ	CHL	CIP	COL	ERY	GEN	LNZ	MEM	QDA	NAL	STR	SMX	TEC	TCY	TGC	TMP	VAN

Part 1A: List of options for location type

Entry	Description
far	Farm
sto	Food store
hom	Home
mar	Outdoor market
pet	Pet store
sla	Slaughterhouse
vet	Veterinary clinic
veh	Veterinary hospital
wil	Wild
lab	Laboratory
unk	Unknown
mix	Mixed
oth	Other

Part 1B: List of options for species

Entry	Description
hum	Human
bov	Cattle
por	Swine
ovi	Sheep
cap	Goat
equ	Horse
rab	Rabbit
fel	Cat
can	Dog
buf	Buffalo
wbu	Water buffalo
rei	Reindeer
mam	Mammal, other
chi	Chicken
duc	Duck
goo	Goose
tur	Turkey
avi	Bird, other
amp	Amphibian
sna	Snake
liz	Lizard
trt	Turtle/Tortoise
rep	Reptile, other
sal	Salmon
tro	Trout
ctf	Catfish
fis	Fish, other
ins	Insect
shl	Shellfish
inv	Invertebrate, other
cam	Camel
fal	Falcon

Part 1C: List of options for animal type

Entry	Description
bre	Breeding
dai	Dairy
egg	Egg-laying
fur	Fur
mea	Meat-producing
pet	Pet
rac	Racing
res	Research
sho	Show
wil	Wild
woo	Wool
wor	Working
zoo	Zoo animal
unk	Unknown
oth	Other

Part 1D: Explanatory note on encoding age

If using:	Use this as entry
Years	1,2,3….
Months	1m, 2m, 3m…..11m
Days	1d, 2d, 3d….30d
Weeks	1w, 2w, 3w…..51w

Age = [Specimen date] - [Date of birth]

Part 1E: List of options for age type

Entry	Description
new	Newborn
you	Young
adu	Adult
unk	Unknown
oth	Other

Part 1F: List of options for age type

Entry	Description
d	Domestic
i	Imported
e	For exportation
u	Unknown
o	Other

Part 2A: List of options for reasons for sampling

Entry	Description
d	Diagnostic
s	Routine screening
spe	Special screening
out	Outbreak investigation
f	Follow-up
l	Laboratory
r	Research
o	Other
u	Unknown
p1	Protocol 1
p2	Protocol 2
p3	Protocol
ash*	AMR surveillance in healthy animals
asd*	AMR surveillance in diseased livestock and poultry
asa*	AMR surveillance in aquatic animals
asae*	AMR surveillance in animal environment

Note: items in **red*** would be good to add in the options, but this is still not possible.

The regional guidelines on monitoring and surveillance of antimicrobial resistance in the food and agriculture sector

The Food and Agriculture Organization of the United Nations (FAO) has embarked on facilitating the development of a series of regionally harmonized guidelines. This was aimed at reinforcing efforts of the animal health sector in the Asia-Pacific region to pursue and contribute to the global strategic objective of strengthening knowledge the evidence base of antimicrobial resistance (AMR) through surveillance and research. This series of guidelines is meant to provide the needed support to key subsectors in food and agriculture, providing guidance that will enable them to generate and contribute relevant, high-quality AMR data to an overarching integrated surveillance. The ultimate outcomes from these individual guidance documents should therefore be viewed beyond the scope for which each guideline is made, that is for the broader purpose it serves - as part of a collective force contributing to generating multi-sectoral, science-based AMR knowledge and evidence that will help define a country's future AMR interventions and actions.

*This Regional Guideline Volume 1 (**Monitoring and surveillance of antimicrobial resistance in bacteria from healthy food animals intended for consumption**) has objectives primarily centered on the interest of protecting public health and provides guidance on the design, planning, implementation, and data management underscores the need to generate AMR data that will reflect an unbiased estimate of prevalence of AMR in target organisms at the human-animal interface.*

Regional Guideline Volume 2 (**AMR surveillance in animal pathogens recovered from diseased livestock**) focuses on the harmonized antimicrobial susceptibility testing of common bacterial pathogens isolated from diseased terrestrial animal commodities in the region (livestock and poultry). The objective for this area of work is to develop treatment guidelines for these common pathogens and in the process, strengthen mechanisms that promote and reinforce good veterinary practices including timely disease diagnosis and evidence-based treatments in lieu of unwarranted metaphylaxis and broad-spectrum preventive treatments.

Regional Guideline Volume 3 (**AMR surveillance in aquaculture settings**) outlines the essential guidance in the conduct of AMR monitoring and surveillance of priority aquatic animal pathogens in the region. As aquaculture is a particularly important resource in the region, a systematic guidance on this area of work from data generation to information sharing will be most valuable.

While the other guidelines will be focused in setting systematic surveillance, the Regional Guideline Volume 4 (**Monitoring bacterial resistance in the animal environment**), will focus on monitoring antimicrobial resistance in bacteria from agriculture settings (such as manure and slurry in livestock farms and aquatic environments) which will leverage the expertise and resources from partner academic and research institutions in the countries. The guideline, while following similar standards where these apply, will be developed with a harmonized and well-coordinated research agenda in mind.

Considering the fundamental need to measure the inputs or exposure that can potentially influence the consequent outcome of antimicrobial resistance, Regional Guideline Volume 5 (**Monitoring antimicrobial usage in animals at the farm level**) is envisioned to support the countries in the region in estimating, documenting, and approximating antimicrobial usage in animals at the field level and is foreseen to complement the country efforts to contribute to the annual global database on AMU by the World Organisation for Animal Health (OIE).

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