Food and Agriculture Organization of the United Nations

Antimicrobial resistance monitoring and surveillance guidelines for food-producing animals and their products in Eastern Africa

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Preparation of this document

This publication is one of the building blocks of the Eastern Africa antimicrobial resistance (AMR) surveillance roadmap that was described in April 2019, by AMR experts from Djibouti, Eritrea, Ethiopia, Kenya, Rwanda, South Sudan, the Sudan and Uganda. The roadmap is presented in Chapter 6 of this document. National AMR experts came together in a regional meeting organized by the Food and Agriculture Organization of the United Nations (FAO), along with other national, regional and international organizations such as the World Organisation for Animal Health (WOAH), International Livestock Research Institute (ILRI), Centers for Disease Control and Prevention in Kenya (CDC), University of Nairobi, Kenya Medical Research Institute (KEMRI), World Animal Protection (WAP) and African Union – Interafrican Bureau for Animal Resources (AU-IBAR).

The aim of the roadmap is to set out the processes, tools, and coordination that technical experts and decision- makers from national governments in Eastern Africa agreed should be undertaken at the regional level to support the development and implementation of national AMR surveillance strategies and plans.

Technical experts and decision makers

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The first building block of the road map concerned the establishment of a regional AMR Surveillance Technical Advisory Group for Eastern Africa (TAG-EA), which was achieved in 2020. The group's first assignment was to develop these regional guidelines for AMR surveillance and monitoring in healthy animals and in their products. The guidelines have been borrowed largely from the Regional *antimicrobial resistance monitoring and surveillance guidelines 1 volume 1: Monitoring and surveillance of antimicrobial resistance in bacteria from healthy food animals intended for consumption,* developed by FAO for the Asia and Pacific region[1]. The lead authors are Rungtip Chuanchuen, Taradon Luangtongkum, Saharuetai Jeamsripong, and Mary Joy Gordoncillo, and it was funded by the United States Agency for International Development (USAID).

These guidelines are the result of a consultative process among regional stakeholders and experts, especially the AMR TAG-EA. The first AMR TAG-EA meeting took place virtually on 15 December 2020. In adapting the guidelines to Eastern Africa, the experts considered the uniqueness of the region in terms of identified priority bacteria, target population for sampling (animals reared for animal source foods), main animal source foods consumed in each country, agriculture sector resources, current capacities (for sampling and for laboratory methods) and available infrastructure. A consultant, Samuel Kariuki of the Kenya Medical Research Institute (KEMRI), adapted the guidelines based on the priorities identified during the AMR TAG-EA meetings.

[1] See FAO. 2019. Regional Antimicrobial Resistance Monitoring and Surveillance Guidelines. Volume 1. Monitoring and surveillance of antimicrobial resistance in bacteria from healthy food animals intended for consumption. Bangkok, FAO. www.fao.org/3/ca6897en/ca6897en.pdf

The draft guidelines were subjected to a first review by AMR TAG-EA national experts in national workshops, which were also attended by members of the AMR coordination committee and the AMR surveillance technical working group. Each country has a multisectoral AMR coordination committee in place and more often has a technical working group on surveillance. Regional and international organizations such as the East Africa Community, Intergovernmental Authority of Development Centre for Pastoral Areas and Livestock Development (IGAD-ICPLAD), ILRI, the African Union, FAO and WOAH also provided their feedback.

The FAO team in Africa – Labia Irène Ouoba, Tabitha Kimani, Joshua Kimutai and Mark Obonyo – provided FAO's input. Finally, additional comments and input from FAO headquarters in Rome were provided by Alejandro Dorado Garcia, Francesca Latronico and Emmanuel Kabali. Supported the adaptation process through the FAO Fleming Fund project. The lead technical team in Eastern Africa included Tabitha Kimani and Joshua Kimutai under the overall supervision of Charles Bebay. UK Aid Direct, the aid agency of the United Kingdom of Great Britain and Northern Ireland (UKAID), supported the adaptation process through the FAO Fleming Fund project.

FAO's Emergency Centre for Transboundary Animal Diseases (ECTAD) office in Eastern Africa, in collaboration with IGAD-ICPALD under the umbrella of the Regional Animal Health Networks (RAHN), will facilitate the adoption of the guidelines by countries and will also monitor their implementation.

Abstract

The animal health subsector within the agriculture sector is the custodian of antimicrobial resistance (AMR) surveillance and risk reduction in livestock, aquaculture, animal products and the animal food production environment. In this publication, *Antimicrobial resistance monitoring and surveillance guidelines for food-producing animals and their products in Eastern Africa*, the emphasis is on safe animal products reaching consumers and on the need to protect public health. These guidelines also provide guidance on the design of AMR monitoring and surveillance, with particular emphasis on harmonized and relevant epidemiology and laboratory methods, as well as AMR data management and reporting. The guidelines underscore the importance of the representativeness of samples to be obtained from apparently healthy animals and from food products of animal origin 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. These guidelines provide a phased- in approach to implementing systems for surveillance and monitoring of bacteria of food-borne origin, starting with collecting easy to obtain samples from abattoirs, moving on to obtaining samples from farms and other sources. These guidelines, focusing on healthy animals and their products, were prioritized in development because this interface represents the most salient pathway towards human exposure from the animal subsector. In addition, these guidelines provide protocols to perform laboratory methods for isolating and identifying selected bacterial species suggested for AMR surveillance and antimicrobial susceptibility testing methods such as disk diffusion, and to interpret laboratory results. They also emphasize and encourage the use of the disk diffusion technique (Kirby-Bauer) for performing antimicrobial susceptibility testing as this test is widely used in most of the laboratories in the region. Among laboratory methods for antimicrobial susceptibility testing, the disk diffusion method is simple, is currently the least expensive option, and is easy to adopt and to perform quality control in a harmonized system of AMR surveillance and monitoring. Other important guidelines for (i) AMR surveillance in bacterial pathogens from clinically ill animals, aquaculture and fisheries, the animal environment (ii) antimicrobial use at the farm level, and (iii) monitoring of residues will be developed through a similar approach.

The guidelines encourage countries to initiate AMR surveillance regardless of their capacity. It also provides guidance and recommended approaches to move progressively towards a regionally harmonized and standardized approach at the outset, which will be important for comparability and monitoring trends and changes in the susceptibility of target bacteria to specific antimicrobials and to inform animal and public health interventions over the coming years. The guidelines are also reinforced by auxiliary AMR surveillance planning tools and standard operating procedures including a common regional template for sampling, AMR laboratory analysis, and AMR data analysis and interpretation.

Contents

Foreword

Antimicrobial resistance (AMR) in microorganisms (bacteria, viruses, fungi, parasites) does not recognize biological, physical or sectoral boundaries. Its potential transmission pathways are best addressed by disciplines that are 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 Food and Agriculture Organization of the United Nation's (FAO) Emergency Centre for Transboundary Animal Diseases (ECTAD) office for Eastern Africa, in collaboration with AMR surveillance experts and regional organizations, have embarked on facilitating the development of a series of regionally harmonized guidelines relevant to this sector.

The *Antimicrobial resistance monitoring and surveillance guidelines for food producing animals and their products in Eastern Africa* is the first to provide 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, these guidelines consider the unique settings of the Eastern Africa 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. These guidelines can also serve as a tool for obtaining regionally harmonized information and bringing benefit to the individual efforts of member countries in the Eastern Africa region. With objectives primarily centred on protecting public health, these guidelines will help showcase the benefits and comparative advantage of a strengthened animal sector in addressing issues at the human–animal–environment interface, and will be valuable for increasing the resilience of livelihoods to threats and crises.

We hope that this publication will further strengthen surveillance strategies in the region and ensure standardized and better AMR surveillance. FAO's ECTAD in Eastern Africa, in collaboration with the Intergovernmental Authority on Development (IGAD), under the umbrella of the Regional Animal Health Networks (RAHN) will facilitate its adoption by countries and will also monitor its implementation.

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FAO also appreciates the Intergovernmental Authority of Development Centre for Pastoral Areas and Livestock Development (IGAD-ICPALD) for their ongoing role in the implementation and monitoring of these guidelines under RAHN.

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Abbreviations

AMR	antimicrobial resistance
AMU	antimicrobial use
AMU/AMC	antimicrobial use/consumption
AST	antimicrobial susceptibility testing
ATCC	American Type Culture Collection
AU-IBAR	African Union – Interafrican Bureau for Animal Resources
BPW	buffered peptone water
CBP	clinical breakpoints
CDC	Centers for Disease Control and Prevention
CEB	campylobacter enrichment broth
CLSI	Clinical and Laboratory Standards Institute
COMESA	Common Market for Eastern and Southern Africa
DNA	deoxyribonucleic acid
EAC	East Africa Community
ECOFF	epidemiological cut-off value
ECTAD	Emergency Centre for Transboundary Animal Diseases (FAO)
EFSA	European Food Safety Authority
ESBL	extended-spectrum β-lactamases
EUCAST	European Committee on Antimicrobial Susceptibility Testing
FAO	Food and Agriculture Organization of the United Nations
FAO AMR PIP	FAO's AMR Progressive Improvement Pathway
FAO-ATLASS	Assessment Tool for Laboratories and AMR Surveillance Systems
GAP	Global Action Plan
IGAD	Intergovernmental Authority of Development
IGAD-ICPALD	Intergovernmental Authority on Development Centre for Pastoral Areas and Livestock Development
ILRI	International Livestock Research Institute
KEMRI	Kenya Medical Research Institute
LIMS	Laboratory Information Management System
MH	Mueller–Hinton
MIC	minimum inhibitory concentration
MRSA	methicillin resistant *S. aureus*
PCR	polymerase chain reaction
RAHN	Regional Animal Health Networks
REC	Regional Economic Communities
SILAB FA	SILAB for Africa
SOP	standard operating procedures
TAG-EA	AMR Surveillance Technical Advisory group for Eastern Africa
TSB	tryptic soy broth
UKAID	UK Aid Direct
USAID	United States Agency for International Development
USDA	United States Department of Agriculture
WAP	World Animal Protection
WGS	whole-genome sequencing
WHA	World Health Assembly
WHO	World Health Organization
WOAH	World Organisation for Animal Health


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Chapter 1 - Rationale and background

1.1. Rationale for the guidelines

Antimicrobials have revolutionized modern medicine, drastically reducing the mortality and morbidity from infectious diseases. Besides human health care, antimicrobials are used in agriculture to prevent and treat diseases in food-producing animals and to promote growth. The widespread use of antimicrobials has hastened the development of antimicrobial resistance (AMR) through increased selection pressure for genes that reduce the effectiveness of antimicrobials such as antibiotics. 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 microorganisms such as bacteria has become a major threat to public health globally and is now considered a rising global concern that affects multiple sectors – human, animal and plant health, and food and the environment.

To address this problem, the World Health Assembly (WHA) adopted the Global Action Plan (GAP) on AMR in 2015, urging Member States to develop their own context-specific, One Health approach based, national action plans on AMR. The Food and Agriculture Organization of the United Nations (FAO), the United Nations Environment Programme (UNEP), the World Organisation for Animal Health (WOAH) and the World Health Organization (WHO) are now coordinating a global approach to address AMR. These guidelines contribute to the second objective of FAO's GAP-aligned action for 2021–2025 (FAO, 2021) on strengthening surveillance and research to support evidence-based decisions. To implement the action plan, FAO is collaborating with the quadripartite and other partners at regional, subregional, national and local levels.

One of the key interventions to address AMR revolves around the development and implementation of AMR surveillance in each country to strengthen the evidence base for effective decision-making and actions at the national level. At the global level, the Quadripartite (FAO, UNEP, WOAH and WHO) is currently working to establish and launch the Quadripartite Integrated System for Surveillance of AMR/AMU, which aims to integrate AMR and antimicrobial use (AMU) information from the four organizations for One Health global advocacy and action. WHO and WOAH, respectively, have operational global systems for the collection and analysis of AMR/AMU data in humans and AMU data in animals. FAO is responsible for generating and sharing global information on AMR in animals and food, and AMU in crop production. However, this process has not been initiated until recently as the focus has been on building the capacities of countries to generate data. The development of the AMR monitoring and surveillance data platform is a key activity of the new FAO Action Plan on AMR 2021–2025 (as approved by the 166th Session of the FAO Council), that was initiated in 2022 with an initial set of participating countries.

Currently, there are several national and regional AMR surveillance programmes involving the animal sector that are operational throughout the world. In Eastern Africa, the countries (Burundi, Djibouti, Eritrea, Ethiopia, Kenya, Rwanda, South Sudan, the Sudan, the United Republic of Tanzania and Uganda) have developed and launched their national action plans to combat AMR, and only a few countries (Ethiopia, Kenya, the Sudan, the United Republic of Tanzania and Uganda) have made some progress towards developing and implementing AMR surveillance programmes under various programmes and donors. In the past, several studies on AMR have been carried out in the region, but the data has not been collated for comparison on the resistant trends, partly due to the lack of a platform to make such a comparison. These guidelines, therefore, seek to provide a common ground for surveillance and monitoring of AMR in food producing animals and in their products in the Eastern Africa region. As AMR surveillance begins progressively in the countries, 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 and contribute to global platforms. International standards for the harmonization of surveillance exist in WOAH terrestrial (WOAH, 2018, Chapter 6.8) and aquatic animal health codes (WOAH, 2021b, Chapter 6.4), and recently, the Codex Alimentarius guidelines for the integrated monitoring and surveillance of food-borne AMR have been approved. However, there are gaps in the practical implementation of these standards and international recommendations. Inadequate implementation has led to a situation where a lack of harmonization in sampling, susceptible testing methods, antimicrobial agents tested, interpretive criteria and reporting often make it difficult to compare data across countries and programmes, which is an essential requirement in increasingly global food chains.

These guidelines build on or contribute to the international standards and recommendations from the WOAH animal health codes and Codex Alimentarius and in subsequent subsections and chapters, they seek to address the practical implementation gaps mentioned in the preceding paragraphs: (i) lack of harmonization in approaches, and (ii) data sharing platform.

1.2. Scope of the guidelines: monitoring and surveillance of antimicrobial resistance in bacteria isolated from healthy food animals

In each country, AMR surveillance systems must: collect data on AMR prevalence, monitor trends and detect the emergence of new resistance. The systems include collecting data from humans, animals, food and the environment. Coordination and harmonization are key to allowing cross-sectoral analyses, which form the basis of identifying priority pathogens and antimicrobials across the sectors. Data on AMU is also important as it constitutes a means of identifying potential overuse, underuse and inappropriate use of antimicrobial medicines, and is the basis for developing interventions to address inappropriate practices (WOAH, 2021).

These regional AMR surveillance guidelines are limited to AMR surveillance in the animal health sector and specifically focus on the surveillance of AMR in bacteria from healthy terrestrial animals intended for consumption. Separate guidelines will be developed or adopted to cover the surveillance of AMR in animal bacterial pathogens (clinical samples), surveillance of AMR in aquatic animals, surveillance of AMR in the animal environment, and surveillance of antimicrobial usage.

These regional AMR surveillance guidelines provide guidance for the development of an AMR surveillance plan for zoonotic and commensal bacteria, underscoring the key elements for harmonized AMR data generation, data collation and reporting of findings, and takes into consideration the unique context of the region. It aims to provide guidelines for a harmonized scheme and protocols for laboratory-based AMR monitoring. The harmonized protocols provide robust science-based technical methodologies from sample collection through data analysis and data reporting as adapted to the regional setting. It provides a quantitative analysis of temporal-based trends in the occurrence and spread of AMR and allows for emerging or specific resistance patterns to be identified.

The protocol was developed following a thorough review of both the current guidance, recommendations, technical specifications and publications (EFSA, 2012b, 2014; WOAH, 2021) and existing national AMR surveillance programmes (EFSA, 2008; Heffernan *et al*., 2011; SSI, SVI and NFI, 2016; NVAL, 2016; USDA, 2014), as it is intended to support the harmonization of a global AMR surveillance system in the region. Although these and other long-standing examples provided the technical framework for these guidelines, the unique setting of the region and practical limitations were also considered. Since 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, these comprehensive

guidelines were reinforced with concomitant initiatives to bridge gaps in knowledge and build essential capacities where AMR and AMR mitigation is concerned. These guidelines also include the experiences and lessons of pilot AMR surveillance initiatives in Africa, with reviews by the FAO Regional Office for Africa (RAF) and members of the AMR Technical Advisory Group of Eastern Africa.

1.2.1. Purpose of surveillance and monitoring of antimicrobial resistance in bacteria isolated from healthy animals

As outlined in the WOAH Terrestrial Animal Health Code Section 6.8.2 (WOAH, 2021a) the purposes of surveillance and monitoring are to:

i) Assess and determine the magnitude, trends and sources of antimicrobial resistance in zoonotic and commensal bacteria;
ii) Detect the emergence of new antimicrobial resistance mechanisms by use of molecular methods;
iii) Provide the data necessary for conducting risk analyses as relevant to animal and human health;
iv) Provide a basis for policy recommendations for animal and human health; and
v) Assess and determine the effects of actions/interventions to combat antimicrobial resistance.

These regional guidelines will further:

a) Assist countries in Eastern Africa to establish baseline data on the prevalence of resistance to antimicrobial agents in zoonotic and commensal bacteria from food-producing animals and their products;
b) Encourage cooperation among member countries;
c) Guide the progressive work of the countries towards producing regionally harmonized AMR data; and
d) Guide on proper use and/or storage for future use any isolates obtained through routine food-borne surveillance programmes (EFSA, 2012b).

1.3. Target countries

These guidelines have been prepared for the following ten Eastern Africa countries: Burundi, Djibouti, Eritrea, Ethiopia, Kenya, Rwanda, Somalia, South Sudan, the Sudan and Uganda. Of those ten countries, eight constitute all the Member States of the Intergovernmental Authority of Development (IGAD), while five are partner states of the East Africa Community (EAC). Eight belong to the Common Market for Eastern and Southern Africa (COMESA). The three Regional Economic Communities (RECs) play a critical role in regional matters including in the health of animals, people, plants and the environment. Considering that the United Republic of Tanzania is the sixth member of EAC, the country's background information is also included in case the country chooses to adopt the guidelines. These target countries can be considered low- and medium- income countries (Table 1.1). Their combined human population is 369 455 130. Ethiopia has the highest population at slightly over 100 million while Djibouti has the lowest (1 million). Burundi and Rwanda have relatively high population densities compared to the other countries. On average about 30 percent of the population live in urban areas.

These countries are encouraged to ensure that their respective initiatives follow a standardized and harmonized protocol for AMR monitoring and antimicrobial susceptibility testing (AST). Considering that the countries targeted have varying capacities to establish and maintain a sustainable surveillance system, a phased approach is recommended. A step wise (phased) approach could

adopt the priorities for surveillance in each country depending on national capacity, concerns and contexts, informed by global guidance. Such phased integrated surveillance systems should connect and build on existing systems to maximize the efficiency of resource use and provide more complete data. To be effective the surveillance and monitoring systems for healthy animals and their food products should both be coordinated and complementary among sectors (public health and animal health, public and private) and levels (local and national).

Table 1.1 Summary of data for selected country indicators

Country	Area (km2)	HDI (2019)	Population (2021)	Population growth (2015–2020)	GNI per capita (2017 USD PPP) 2019	Population density
Burundi	27 834	0.433 (185/189)	12 255 433	3.15	754	440.30
Djibouti	23 200	0.524 (166/189)	1 002 187	1.56	5 689	43.20
Eritrea	117 600	0.459 (180/189)	3 601 467	1.18	2 793	30.62
Kenya	580 367	0.601 (143/189)	54 985 698	2.32	4 244	94.74
Rwanda	26 338	0.543 (160/189)	13 276 513	2.61	2 155	504.08
Somalia	637 667	0.258 (170/189)	1 544 906	2.83		2.42
Sudan	1 886 000	0.51 (170/189)	44 909 353	2.39	3 829	23.81
South Sudan	1 037 000	0.433 (185/189)	11 381 378	0.87	2 003	10.98
Uganda	241 037	0.544 (159/189)	47 123 531	3.59	2 123	195.50
United Republic of Tanzania	945 087	0.529 (163/189)	61 498 437	2.97	2 600	65.07

Note: HDI = human development index. Country position globally is shown in brackets.

1.4. Livestock species and animal source foods to which the guidelines apply

Annex 2 shows the livestock population figures for the years 2015 to 2019 (FAOSTAT, 2021). Cattle, chickens, goats and sheep are the common species found in all eleven countries. Pigs are important in Burundi, Kenya, Rwanda, the United Republic of Tanzania and Uganda, and fewer are reared in Ethiopia and Somalia. Djibouti, South Sudan and the Sudan do not rear pigs. A significant number of camels are found in Ethiopia, Kenya, Somalia and the Sudan. Cattle are raised in very heterogenous systems that include pastoral/agropastoral, mixed crop–livestock systems and market oriented dairy systems of varying scales and intensification. Sheep and goat are mainly raised in pastoral/agropastoral and mixed crop–livestock systems. Poultry are also raised in varying production systems ranging from free range to large scale intensive. There is limited data available on the proportion of animals raised under each system. According to FAO (2019a and 2019b), most poultry is raised under extensive systems (55 percent in Uganda and 48 percent in Kenya) followed by semi-intensive (32 percent in Kenya and 20 percent in Uganda) and then intensive (25 percent in Uganda and 20 percent

in Kenya). Most of the cattle (72 percent in Kenya, 91 percent in Uganda) are raised in pastoral and agropastoral systems. In Kenya, 23.8 percent are raised under dairy systems (extensive to intensive) while a small proportion of cattle (4.2 percent) produce meat through ranching or feedlot systems.

The guidelines should be used for these major food animal species including poultry (broilers, layers and free range), swine, cattle, sheep and goats and their products: meat (chicken, pork, beef); eggs and milk

Annex 3 presents the proportion of live animals slaughtered annually in Eastern Africa, while Table 1.2 summarizes the volume of meat produced annually (average from 2015 to 2019) and the proportionate contribution from each species. In all countries, beef accounts for the highest proportion (31 to 68 percent). The second and third most produced live animal differed across countries, but generally included: goat (Burundi, Djibouti, Eritrea, Ethiopia, South Sudan and the United Republic of Tanzania); chicken (Kenya, Rwanda, Uganda and the United Republic of Tanzania); pork (Burundi, Uganda); sheep (Eritrea, Ethiopia, South Sudan); camel (Djibouti, Kenya, Somalia, the Sudan); game meat (Ethiopia). In all countries, except Somalia, cattle account for the highest contribution (56 to 100 percent) of milk (Table 1.3). In Djibouti, Kenya and Somalia, camels account for a significant proportion of milk produced. The volume of eggs produced is shown in Table 1.3.

Table 1.2 Total meat produced and proportionate contribution from each species

Country	Total meat production of all species (tonnes)	Proportionate (%) contribution of each species to total meat production (based on 2015– 2019 average)						
		Chicken	Cattle	Goat	Pig	Sheep	Camel	Game
Burundi	42 6314	16	**35**	**22**	**25**	2	snr	nd
Djibouti	15 293	nd	**41**	**15**	snr	14	**30**	nd
Eritrea	40 912	3	**54**	**15**	snr	**16**	11	nd
Ethiopia	684 811	10	**50**	**12**	0.3	**13**	4	**12**
Kenya	750 756	**10**	**63**	6	2	4	10	4
Rwanda	105 023	**17**	**31**	**19**	12	5	snr	15
Somalia	188 901	2	**29**	20	snr	**23**	**25**	nd
Sudan	969 452	7	**39**	12	snr	**27**	**15**	nd
S. Sudan	415 719	5	**54**	12	snr	**29**	snr	nd
Uganda	413 544	**16**	**42**	9	**31**	2	nd	nd
United Republic of Tanzania	562 938	**14**	**68**	7	3	4	snr	4

Source: computed from FAOSTAT (2021) FAOSTAT: Production: Crops and livestock products. In: FAO. Rome. Cited May 2021. https://www.fao.org/faostat/en/#data

Notes: nd = no data available from FAOSTAT; snr = species not reared; bold and red = species among the top three contributors of local production of meat.

Table 1.3 Milk (including proportionate contribution of each species) and egg production

Country	Total milk production of all species (tonnes)	Proportion (%) contribution of each species to total milk production				Total egg production of chickens (tonnes)
		Cattle	Goat	Sheep	Camels	
Burundi	118 354	**56**	**43**	1	0	62 000
Djibouti	14 896	**59**	0	0	**41**	nd
Eritrea	159 434	**72**	**12**	8	8	51 562
Ethiopia	3 539 076	**90**	2	2	5	1 189 245
Kenya	5 019 160	**75**	5	2	**18**	1 670 490
Rwanda	249 704	**67**	**29**	3	0	217 120
Somalia	2 157 458	20	17	**18**	**44**	63 809
Sudan	4 545 200	**64**	**25**	9	1	1 043 333
S. Sudan	3 293 719	**81**	**14**	5	0	nd
Uganda	1 735 408	**100**	0	0	0	954 696
United Republic of Tanzania	2 425 185	**91**	9	0	0	1 889 627

Source: computed from FAOSTAT (2021) FAOSTAT: Production: Crops and livestock products. In: FAO. Rome. Cited May 2021. https://www.fao.org/faostat/en/#data

Notes: nd = no data available from FAOSTAT; bold and red = 1 to 3 species cumulatively contributing at least 80 percent of local production of milk.

Data also shows that some countries in the region imported substantial amounts of animal source foods, which can also be included in the surveillance. Products imported into the countries to decreasing volumes include milk, poultry meat, eggs, pig meat and bovine meat. The main importers of milk include Djibouti, Kenya and the Sudan (Table 1.4). The main products exported to decreasing volumes include milk, mutton and goat meat, bovine meat and others. The main exporter of milk is Uganda, while the major exporter of beef is the Sudan. Ethiopia exports the greatest volume of mutton and goat meat.

Table 1.4 Animal source food (1 000 tonnes, average 2015–2018) imported into or exported out of Eastern African countries

Country	Poultry Meat		Eggs		Milk		Bovine Meat		Mutton & Goat Meat		Pig Meat		Other Meat	
	Imp	Exp	Imp	Exp	Imp	Exp	Imp	Exp	Imp	Exp	Imp	Exp	Imp	Exp
Djibouti	2.5		2		13.75									
Ethiopia	0.5				1.75			1.5		16.75	0.5			
Kenya	0.25		2		96.5	1.75	0.5	1.5		4.5	1	2.25		4
Rwanda					1.75	1								
Sudan	1		0.75		34.25		0.5	9.75		3				
Uganda		0.25		2	1.25	79.5								
United Republic of Tanzania	1.25				6.75		0.75	0.75		1.75	0.75			1

Source: computed from FAOSTAT (2021) FAOSTAT: Production: Crops and livestock products. In: FAO. Rome. Cited May 2021. https://www.fao.org/faostat/en/#data
Notes: Imp= Imported ; Exp = Exported

Per capital consumption data is also important in designing AMR surveillance programmes in each country. The per capital consumption of meat varies (7 kg in Ethiopia; 6 kg in Uganda and 11.3 kg in Kenya).
The per capital milk consumption also varies (121 litres in Kenya: 36 litres in Uganda and 19 litres in Ethiopia). Data for other countries was unavailable.

1.5. Target bacteria to which the guidelines apply

From a public health perspective and, therefore, surveillance of AMR in healthy animals (cattle, camels, sheep, goats, chickens, pigs) and their products (meat, eggs, milk) food-borne pathogens *Salmonella enterica serovar Typhimurium* and *Enteritidis* and *Campylobacter spp*. (*C. jejuni and C. coli*) and the commensal bacteria *Escherichia coli and Enterococcus spp.* (*E. faecium and E. faecalis*) and *Staphylococcus aureus* (See Chapter 2, Section 2.6) are those considered most relevant to AMR monitoring in Eastern Africa.

1.6. Towards regional harmonization and standardization

These guidelines allow countries, in a phased approach, to progressively pursue the ideal sampling, sample collection, laboratory methods and data management relevant to AMR surveillance. Based on regional livestock populations, the livestock products that are most consumed and keeping current AMR capacity in mind, Chapter 2 highlights priority pathogens, livestock species and sampling points for entry into regional harmonization. In summary, 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 Eastern Africa (Table 1.5). 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 in a truly One Health approach while following the Codex guidelines for integrated surveillance of food-borne antimicrobial resistance (https://www.fao.org/fao- who-codexalimentarius/sh-proxy/en/ adopted in 2021).

Table 1.5 Baseline entry points and subsequent targets for regional harmonization on surveillance of antimicrobial resistance in food-borne microorganisms from healthy food animals and their products

REGIONALLY HARMONIZED ANTIMICROBIAL RESISTANCE SURVEILLANCE		
	BASELINE ENTRY POINTS FOR INITIATION 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 carries out its routine AMR surveillance.
TARGET POPULATION	AMR data are obtained from bacteria from the most accessible population of animals and animal food products. Broiler chickens are adopted as the entry species in Eastern Africa.	Prioritize AMR data from bacteria obtained from the main known food producing animal species contributing to the most consumption yield in the country. Broiler chickens, cattle/beef.
SAMPLING STRATEGY	AMR data are obtained from a convenient number of samples and based on accessibility to these animals in Eastern Africa. 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.	Takes into account both epidemiologic (e.g. representativeness) and biologic (e.g. type of sample, timing) considerations, as well as the feasibility of logical support for implementation.
TARGET BACTERIA	Starts with targeting *E. coli* and *Staphylococcus aureus* considering the available resources and capacity. If there is an operational food-borne zoonoses surveillance programme (e.g. *Campylobacter* spp., *Salmonella* spp.) consider including them but give particular attention to the context from which and how and when the isolates were obtained when drawing conclusions.	Both zoonotic/food-borne (*Salmonella* spp. and *Campylobacter* spp.) and commensal (*E. coli, Enterococcus* spp. and *Staphylococcus aureus*) organisms are included in the routine AMR monitoring and surveillance.
TYPE OF DATA GENERATED	Qualitative data are obtained through disk diffusion with consideration for the latest international standards. The value and validity of the resulting data may be compromised and will have limited use for an AMR surveillance programme.	Quantitative data using Minimum Inhibitory Concentration (MIC) following international standard methods are generated, reported and stored.
PANEL OF ANTIMICROBIALS	A few select antimicrobials (highlighted in Chapter 4) are included in the panel. The appropriate highest priority critically important antimicrobials are preferred.	The core panel of antimicrobials monitored is harmonized with that of the region, as per CLSI guidelines and as detailed in Chapter 4.

Chapter 2 - Sampling

2.1. Introduction

Effective surveillance of AMR in zoonotic and commensal bacteria from healthy food animals and their products provides an estimate of the national prevalence of AMR at the farm level (or at the retail level) for different bacterial/antimicrobial combinations (e.g. the proportion of farms that have AMR for the given combination). Cumulative data from the surveillance are accepted as providing meaningful information to guide evidence-based actions/interventions to address AMR. Such information has the potential to transform policies and practices and should be based on accurate data representing the population targeted. 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. In addition to the guidelines below, countries are advised to refer to sampling methods such as those described by the European Food Safety Authority (EFSA) and summarized in EFSA (2020).

2.2. Target population of food-producing animal species

While Chapter 1 provides background data on livestock populations, volumes of animal source foods produced, exported and imported, the countries should continuously examine how their livestock value chains evolve (based on existing national livestock databases, such as FAOSTAT (http://www.fao.org/faostat/en/#data) and assess which sources are likely to contribute most now (and in the future) to a potential risk to animal and human health (WOAH, 2021a, Section 6.8.4). As seen in Chapter 1, cattle, poultry (layers, broilers and indigenous/local), sheep and goats, and swine and camels are the major sources of animal food and products in the region. While more quantities of red meat (from cattle, sheep, goats) are consumed in the region compared to poultry meat and pork, the latter two are likely to be the main sources for AMR followed by beef. In the case of poultry, in urban and peri-urban areas, spent layers and broilers are the most consumed and produced in intensive smallholder farms characterized by low levels of biosecurity, hence the pressure to use antibiotics to keep them healthy. However, the most common source of chicken meat in rural areas are free-range indigenous birds. The broilers are often slaughtered at the farm level or at live bird markets under unhygienic conditions. Therefore, bacterial isolates from poultry (beginning with broilers) and cattle are prioritized for regional harmonization, while the other species should be targeted depending on the country's preferences and situation. The selection can be expanded as necessary in accordance with the country's needs, access to available resources, and value in their desired outcomes for AMR surveillance. Progressively, including all chicken species (broilers, layers, indigenous) will provide the basis for comparison in terms of bacterial pathogens isolated and their AMR patterns. 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.

Considering that live animal imports are minimal (or there is inadequate data on formal trade flows) compared to the local production and that domestic production can be linked to antimicrobial use, the monitoring should primarily focus on animals raised for domestic consumption.

2.3. Target food of animal origin

In addition to samples from live healthy animals mentioned in Section 2.2, the corresponding animal source foods to be targeted in the AMR surveillance include poultry meat, beef, other red meat (sheep, goats, camels), milk (dairy cattle and camels) and eggs. While selection of these commodities should reflect consumption patterns in each country, the agreed entry points for regional harmonization

is broiler meat and beef. As chicken, pork and beef are the types of meat most consumed in the region (Vivien, 2004) and pork is consumed in Burundi, Kenya, Rwanda and Uganda, the region may consider including pork as the third commodity for regional harmonization. Considering that imports of animal source foods are minimal compared to local production and that domestic production can be linked to antimicrobial use, the monitoring should primarily focus on target products domestically produced to assess the source of AMR in the country.

2.4. Target imported food of animal origin

Regardless of how the livestock value chains evolve, it is expected that imports of animal source foods will increase but vary in each country in terms of products and volumes. AMR monitoring will be expanded to initially include imported eggs, milk and meat, which are often traded across borders in the region. This is complementary monitoring that should be analysed and reported separately from the results for domestically produced eggs. Net importing countries in the region such as Djibouti may choose some imported products as their priority for AMR surveillance.

2.5. Sampling points, sites and samples

Sampling should focus on live animal populations preferably close to or at slaughter as they represent the risk to consumers. 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. Table 2.1 summarizes the merits and demerits of each sampling point while Table 2.2 summarizes the sampling site and samples.

Table 2.1 Merits and demerits of various sampling points

Sampling point	Merits	Demerits
Farm level	a. ability to obtain sufficient quantities; b. easy to randomize the sampling units to obtain a more objective view; c. availability of the right person to answer the questionnaire; and d. ability to observe without verbal communication.	a. non-cooperating farm owners/managers; b. logistical challenges of accessing many farms and associated costs – the terrain to assess the farms may be challenging; and c. bad weather may inhibit the sample collection to involve the required number of farms per day.
Live animal market	a. obtaining pooled samples is easy; b. the right population /sample size can be achieved with convenience; and c. most markets are easily accessible hence time and resource saving.	a. risk of spread of infections is high; b. inability to contact trace the origin of a disease; and c. resistance from the sellers/vendors.
Slaughterhouse	a. easy to swab animals and carcasses, persons and the environment; b. randomized sampling can be easily achieved; and c. a wide range of samples are easy to obtain.	a. high risk of contamination, thus may not provide a true picture; and b. animal owners may be uncooperative.
Supermarket	a. ability to contact trace to the farm owners; and b. ease of random sampling.	a. resistance from management.

For regional harmonization, the priority sampling point for live broiler, broiler meat and live cattle and beef is the slaughterhouse or slaughter point. It should be noted that most broilers in Eastern Africa are slaughtered at the farm level (though few slaughterhouses exist in some countries) and are delivered directly to supermarkets, retail outlets and hotels. In this case, the slaughter point may be the same as the farm, and therefore the demerits of the farm as a sampling point applies.

Table 2.2 Sampling sites and samples

Animal	Sampling site	Sample	Sampling point *
Poultry	Cloaca	Ceacal content	**Slaughterhouse/slaughter point,** live bird markets, farm
	Meat (neck, breast, skin)	Swabs	Slaughterhouse/slaughter point
	Layer poultry house	Boot swab	Farm
		Eggs	**Supermarkets**, wholesale market, farm
Cattle, goats, sheep, camels	Recta	Rectal content/ faeces	
		Milk	**Collection points**, farm
		Meat (beef, mutton)	**Slaughterhouse/slaughter point**, supermarkets, retail butchers
Swine	Recta	Rectal content	Slaughterhouse/slaughter point
	Snout/nose	Nasal swab	Farm
		Pork	**Slaughterhouse/slaughter point**; retail butchers

*where more than one sampling point is identified, the priority for regional harmonization is in **bold**

2.5.1. Food animals sampled at the farm

Faecal and cloacal samples may be collected at the farm to monitor AMR. Although data from isolates obtained at this level of the chain allow for a more accurate assessment of the impact of antimicrobial exposure of the source animals, as mentioned in Table 2.1, the 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, for laying hens sampling at the farm level (e.g. using boot swabs) will likely remain the best approach since layers are not routinely slaughtered like broilers or improved indigenous chickens. Nasal swabs for swine herds should be obtained at the farms. Animal food products (milk and eggs) will also be sampled at the farm (production point). Small scale farming villages/settlements could be used as proxies for the farm or slaughterhouse sampling where sampling frames are lacking.

2.5.2. Food animals at slaughter

This is the point where livestock are closest to consumer exposure. It is also usually the most convenient and cost effective point for collecting animal samples, hence it is the preferred entry point for regional harmonization.

For most livestock in the region, there are numerous 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, meaning unrefrigerated, to consumers. For poultry, small-scale slaughter points predominate.

These are often makeshift slaughter areas linked with live bird markets. In such cases, live poultry from live bird markets may be targeted for sampling.

As mentioned in Table 2.2, samples that may be collected from slaughterhouse/slaughter points include meat/carcass swabs, faecal–cloacal samples/rectal content and nasal swabs. Fresh meat can be collected either at the carcass cutting plant or in slaughterhouses. Swabs from the slaughter surfaces should also be taken to check for possible contamination and to verify effective decontamination of the slaughter slabs.

2.5.3. Sampling animal products at collection/cooling and processing plants

Bulk milk samples can be collected from milk collection centres. The samples collected can be for both the individual farmer (farm level bulked) and community (several farms bulked). Community bulked samples can also be collected from the cooling and processing plants.

2.5.4. Sampling animal products at the supermarket, wholesale or retail stores

Meat, milk and eggs can also be sampled at retail outlets. Sampling fresh meat at the carcass cutting plant as well as milk from collection points and eggs from farms may make it easier to differentiate between the domestic and imported products. At the supermarket, wholesale and retail outlets, aside from issues of potential cross contamination, domestic and imported raw meat, either fresh or frozen, milk and eggs 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 the exposure of consumers to resistant bacteria.

2.6. Bacterial species to be monitored

The monitoring of AMR in food-producing animals and food products of animal origin should cover commensal bacteria and food-borne zoonotic bacteria, as per the WHO priority list (WHO, 2015; 2017) and WOAH (WOAH, 2021a, Article 6.8.5). Prior to 2017, the selection of pathogens for research and development activities had been largely guided by pharmaceutical companies. This was rather subjective. In 2013, a list was issued by the United States of America Centers for Disease Control and Prevention CDC, 2013), and another list by the Public Health Agency of Canada in 2015 (Garner *et al.*, 2015). WHO was thus requested by Member States to develop a global priority pathogens list (global PPL) of antibiotic-resistant bacteria to help in prioritizing R&D of new and effective antibiotic treatments. For its part, WOAH has identified the same categories of bacteria that may be included in surveillance and monitoring programmes. The five priority pathogens for these guidelines have been picked from the list and have also been adopted for surveillance and monitoring of AMR in other regions. The five commensal and enteropathogenic bacterial species are selected based on their capacity to serve as a reservoir of multi drug resistance genes/markers, public health significance, national/regional/international trade impact, and relatively easy to culture and isolate in a moderate laboratory facility. These are the five species, in order of priority for regional harmonization.

- *E. coli* is a commensal bacteria (normal flora), however, as it quickly adapts to different host/environments it easily transfers plasmids/resistant genes to other microbes. It is, therefore, a good indicator organism for drug resistance.
- *Staphylococcus aureus* is a skin commensal, however, when it contaminates milk or meat and enters the food chain it causes lethal infection to humans. It has exhibited methicillin and vancomycin resistance, thus proving difficult to treat.

- *Enterococcus* spp., which is a commensal, has also developed resistance to vancomycin at an alarming rate in recent decades.
- *Salmonella* spp. and *Campylobacter* spp. are pathogenic zoonoses. They have shown resistance to fluoroquinolones.

2.6.1. 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. Commensals are exposed to antimicrobials 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.

2.6.1.1. Commensal *E. coli*

Commensal organisms give an indication of resistance genes that could enter or be in the food chain (Lambrecht *et al.*, 2019). Resistance in *E. coli* collected from food (primarily meat) can be compared with *E. coli* in faecal samples from animals to determine whether the introduction of AMR in the food chain is likely to originate on the farm or from subsequent processing of animal products. As commensal organisms do not cause clinical illness, sampling for commensal *E. coli* requires an active surveillance programme (Tello *et al.*, 2020). Enteropathogenic *E. coli*, is not a normal flora in food animals; therefore, if it is isolated from apparently healthy animals, it should be an indicator of ill-health and the animal should not be let into the food chain. Levels of resistance in commensal *E. coli* can be compared to resistance found in pathogenic *E. coli* isolated from clinical cases in humans or animals to facilitate the risk assessment of the food chain, and to better understand the potential transmission pathways for resistance.

2.6.1.2. *Staphylococcus Aureus*

The gram-positive bacterium *S. aureus* was identified as a priority both in animals and humans, even though strains that cause disease in each are different (Bouchami *et al.,* 2020). In animals, it is of concern as it is implicated as a primary causative agent of bovine mastitis. In humans, methicillin resistant *S. aureus* (MRSA) in milk would be a major public health risk for causing disease. The emergence of MRSA is the cause of morbidity and mortality in both community and hospital acquired nosocomial infections (Holten Møller *et al.*, 2020). MRSA has recently been observed in Kenya from the clinical samples and normal samples (e.g. milk) of animals (Omwenga *et al.,* 2020).

2.6.1.3. *Enterococcus Faecium* and *E. Faecalis*

The genus *Enterococcus* is ubiquitous in nature and a number of species can be found in a range of habitats. *Enterococcus* spp. are also common members of the normal gastrointestinal (GI) flora of both livestock and humans. It is an important indicator for the commensal bacteria for AMR studies.

E. faecium is present in the faeces and is easily spread from animals to humans through contamination of the meat by the faeces or lack of proper hygiene. This organism is inherently tenacious, and it has built a resistance to antibiotics that has allowed it to thrive in most environments. The continual use of antibiotics in animal husbandry has enabled *E. facium* to develop a resistance to vancomycin. *E. faecalis* being a commensal may not cause harm to healthy individuals. However, it can enter the body through wounds, blood or urine and cause disease, particularly to hospitalized individuals because of reduced immunity. *E. faecalis* has both natural and acquired immunity from antibiotic treatment. It is commonly transmitted due to poor hygiene or contamination from faecal matter.

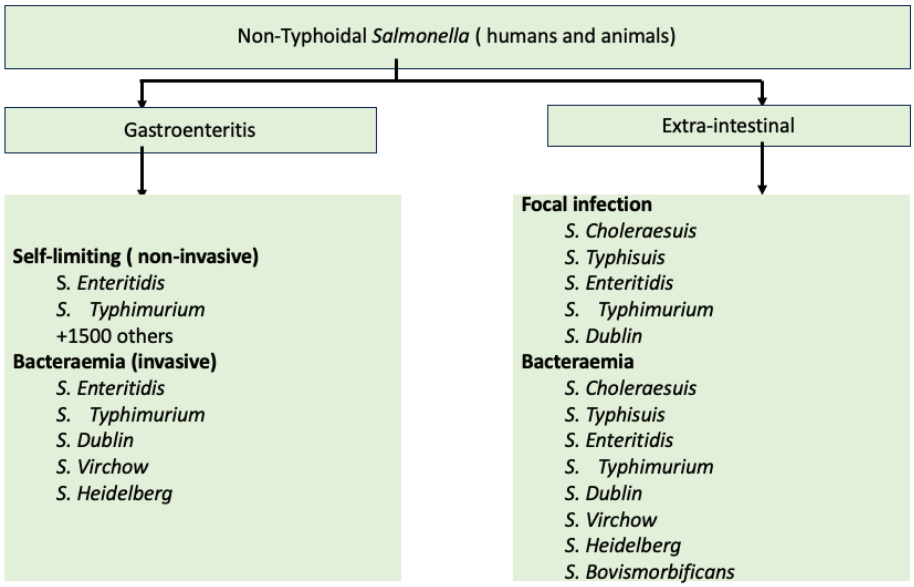
2.6.2. Food-borne (zoonotic) bacteria

Food-borne bacteria (*Salmonella spp.* and *Campylobacter spp.*) are bacteria occurring in animals and causing foodborne 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 because of better or successful control programmes. Therefore, commensals are likely to be important in AMR monitoring for better comparison in the future.

2.6.2.1. *Salmonella* spp.

Salmonella was selected because it is a food-borne bacteria that can be found in animals, food and humans (Wilson *et al.*, 2020). Food animals are mainly carriers of this pathogen. Harmonized surveillance of *Salmonella* spp. permits comparisons of the prevalence of resistance in bacteria obtained from different species and sources and aids in risk a ssessment as well as providing a better understanding of the transmission of resistance. Salmonellae are isolated from clinical samples from sick humans and animals, via passive surveillance programmes, as well as from healthy animals and from foodstuffs (of animal and non-animal origin) via active surveillance programmes. Figure 2.1 summarizes zoonotic non-typhoid Salmonella serotypes.

Figure 2.1 Zoonotic non-typhoidal salmonella serotypes



2.6.2.2. *Campylobacter* species

Campylobacter spp. is a gram-negative bacteria. *Campylobacter* typically appear as comma- or s-shaped and are motile. It is mostly found in chicken carcasses. *Campylobacter* infection may be due to eating raw or undercooked poultry. Antimicrobial resistance in *Campylobacter jejuni* and *coli* are rising, thus the organism is considered an important zoonotic bacteria.

2.7. Summary of bacteria, species, sampling points, sites and samples

Where possible, all five bacteria should be included in the surveillance programme (Table 2.3).

Table 2.3 Recommended combination of sampling points, bacterial species and sample type for surveillance of antimicrobial resistance in different livestock species and their food products

Animals	**Place of sample collection**	**Bacterial species**				
		Salmonella	***E. coli***	***C. coli & C. jejuni***	***E. faecium & E. faecalis***	***Staphylococcus aureus***
Broiler	Farm	Boot swab	Cloacal swab	-	-	-
	Slaughterhouse	Caecum	Caecum	Caecum	Caecum	-
Layer and indigenous chickens	Farm Live bird market Hatcheries	Boot swab Eggs	Cloacal swab Caecum Eggs	Caecum	Caecum	Boot swab
Chicken meat	Slaughterhouse retail outlet	Skin/ meat swab	-	Skin/ meat swab	-	Skin swabs
Cattle	Farm live animal market	Faecal	Faecal	Faecal	Faecal	Milk Hand swabs of Milkers
Beef	Slaughterhouse supermarket/ Butchery/ retail outlet	Meat	Meat	-	Meat	Meat
Pigs	Farm	Faecal	Faecal	Faecal	Faecal	Nasal swab
	Slaughterhouse	Caecum	Caecum	Caecum	Caecum	Meat
	Retail outlets	Meat swab	Meat swab	Meat swab	Meat swab	Meat swab
Sheep and goats Camels	Farm live animal market	Faecal	Faecal	Faecal	Faecal	Milk
Sheep and goats Camel meat	Slaughterhouse, supermarket/ retail outlet	Meat	Meat	-	Meat	Meat
Environmental samples		Boot swabs Feeds Water	Boot swabs Feeds Water	Feeds Water		

Note: 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 apparently healthy animals only.

Considering that resources and capacities may not allow immediate implementation of all the recommended combinations as summarized in Table 2.3, each country is encouraged to follow the suggested order to meet both the aspirations of regional harmonization and national surveillance objectives. Based on capacity assessments/baseline entry points, the countries will join the regional harmonization by setting up an active surveillance programme for *E. coli*. The first priority target species is poultry (specifically broilers) sampled at a priority sampling point at slaughter, where neck, skin and breast pooled swab samples as caecum will be collected. The second priority sampling point is the farm and cloacal swabs, and boot sample (environment) will be collected. The third priority sampling point will be at retail (supermarkets) where the breast meat sample is recommended.

The second priority target species after broilers is cattle where beef and intestinal contents are recommended samples sourced at slaughter. For the species and samples, countries should decide on the order of priority for inclusion depending on the national surveillance goals, human resource capacity and laboratory infrastructure and supplies.

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.

2.8. Sampling frame

The sampling frame is a list of sampling units within the target population from which samples can be collected. Each country working closely with an epidemiology team and using livestock population and production data in Chapter 1 can choose to develop or identify an appropriate sampling frame for their identified target population. This can be a list of farms (categorized by intensive, semi-intensive, extensive, small, medium and large scale), slaughterhouses, or other sources suitable for sampling. Ideally, the sampling frame should coincide with the target population (i.e. both have the same number of sampling units representing the whole population, e.g. all farms in the country). Therefore, sampling frames would be made to include the maximum possible sampling units from the target population (i.e. 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 emphasized 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 country.

2.8.1. Sampling frame for farms

The epidemiological unit for broilers, sheep and goats is the flock, for pigs and cattle it is the farm, and for camels it is the herd. 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.

2.8.2 Sampling frame for slaughterhouses

The sampling frame should include slaughter facilities that account for at least 80 percent of the slaughter population of the food animal species prioritized. A complete list of all slaughter points 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.

2.8.3. Sampling frame for retail outlets

The sampling areas (or regions) should account for at least 80 percent of the national retail outlet population. The number of different meat, milk and eggs categories to be sampled is assigned proportionally to the size of the retail outlets serving the human population in the areas.

2.8.4. When a suitable sampling frame is not available

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

2.9. Sample size calculation

Various references and tools are available and can be used for sample size calculation (some examples include: Epitools, http://epitools.ausvet.com.au/content.php?page=SampleSize; and Open Epi, http://www.openepi.com/SampleSize/SSPropor.htm).

However, it should be noted that this part of the planning can also benefit from the input of the relevant units of the competent authority in this area of expertise (e.g. the epidemiology section of the veterinary department). For each country, the laboratory teams should work closely with epidemiology units for efficient field surveillance. The epidemiologists, laboratory experts and stakeholders should also make careful investigations into the convenience and safety of the laboratory team to collect samples especially at the farm level and in live animal markets, giving guidance on the samples to be collected in every field visit and at what interval. Whatever approach is used, it should reflect the set objectives for the national AMR surveillance, and comply with the WOAH Terrestrial Animal Health Code Section 6.8.4 (WOAH, 2021a) 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.
- The sample should avoid bias and be representative of the animal population, process, product or other unit of interest while 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 the 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.

2.9.1. 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 resistant phenotypes circulating in animal populations are present in these species.

These indicator bacteria are important to monitor because: (i) commensal bacteria that contaminate food such as Staphylococcus aureus 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 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 the sample size for this approach are shown in Annex 4.

2.9.2. 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 use 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.

(i) Sample size calculation for active surveillance of food-borne zoonotic bacteria
The same approach in Steps 1 to 3 described in Annex 4 for commensal bacteria may be carried out to establish the prevalence of resistance among food-borne pathogen isolates (i.e. *Salmonella* spp., *Campylobacter* spp. and *Staphylococcus* aureus) 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 the sample size for this approach are presented in Annex 5.

(ii) 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; 2012a; 2014) which made the following assumptions: (a) infinite population size for the number of bacteria isolates in each study population and country; (b) confidence level of 95 percent and a power of 80 percent; and

(c) perfect sensitivity and specificity of the diagnostic test (antimicrobial susceptibility testing). 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 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 of 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** Currently there is minimal active national surveillance programme in the Eastern African countries. In some countries, e.g. Kenya, the private sector on large scale poultry farms has 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.

(ii) **The use of existing active surveillance for antimicrobial susceptibility testing 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 also be taken into account, and where the sampling is limited, the nature of the sampling should be taken into consideration when interpreting the results.

2.10. 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 designed carefully to generate a statistically sound, unbiased estimate of the national prevalence of AMR, but the implementation also needs to be sufficiently practical and cost effective if such activities are to be sustainable. These considerations should be underscored in the design development.

2.10.1. 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 or apparently healthy food animals. Sick animals should not be part of this surveillance component (and should not enter the food chain).

2.10.2. Emphasis on domestic production

This is particularly important if the AMR surveillance data is primarily intended for policy making and recommendations on antimicrobial use 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 be reflected accordingly in the sampling plan. Results of the surveillance can be put into context with the resistance situation in people.

2.10.3. Sampling by probability proportional to size

The number of samples collected in each stratum (e.g. farms, slaughterhouse, retail outlets or geographic location) should be proportional to the size of the respective stratum in the sampling frame (e.g. strata of farms categorized by intensive, semi-intensive, extensive, small medium and large scale). This allows better representation of the strata as these are represented in accordance with their proportional share in the overall population.

2.10.4. Sampling frequency

The sampling should be performed annually, multiple times, with, ideally equal distribution over the year. Distributing the collection throughout the year enables different seasons to be covered, allows for the 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 to optimize the use of resources in the case where they are too limited to cover all species each year.

2.10.5. 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 2.4 are considered when selecting recovered target isolates for antimicrobial susceptibility testing (AST).

Table 2.4 Sampling plan for bacterial species for antimicrobial susceptibility testing

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.
Enterococcus faecium and *E. faecalis*	Only one isolate/bacterial species from the same epidemiological unit per year.
Staphylococcus aureus	Only one isolate from the same epidemiological unit per year.

*Isolates mainly come from pooled samples collected at the same epidemiological unit (e.g. farm).
**Epidemiological unit means a group of animals with a defined epidemiological relationship that share approximately the same likelihood of exposure to a pathogenic agent.

2.10.6. Streamlining the 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 normative routine operations within the system and help make the overarching efforts more relevant across the animal health sector.

2.10.7. Non-random sources in monitoring and surveillance of antimicrobial resistance

Although population-based AMR surveillance regularly conducted over time allows for an 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 analyse AMR, trends need to be interpreted with caution. Furthermore, such data should be generated using harmonized laboratory protocols.

Chapter 3 - Sample collection and transport

3.1. Introduction

When 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 that will address objectives as set by the country. Prior to taking samples, careful consideration should be given in defining the purpose for which the specimen is required. This will determine the type and number of samples needed to provide valid results. When samples are taken from live animals (e.g. at the farm), care should be taken to avoid injury or distress to the animal or danger to the attendants. When handling biological material, the risk of zoonotic disease should be kept in mind and precautions taken to avoid human infection. Care should also be taken to avoid environmental contamination, or risk of spread of disease through insects or fomites. The technician should ensure that the right specimen and correct amount is collected and sent to the laboratory.

3.2. Samples to collect for animal–bacteria combinations

As a starting point, countries may consider the recommended combination of zoonotic (i.e. *Salmonella, Staphylococcus aureus* and *Campylobacter*) and commensal bacteria (i.e. *E. coli* and *Enterococcus*) in food animals as detailed in Table 2.3. For these guidelines and guided by Table 2.3, the focus will be on the following veterinary sample types:

(i.) cloacal swabs/caecum content/faeces
(ii.) nasal swabs
(iii.) boot swabs
(iv.) animal products
  a. meat
  b. milk
  c. eggs.

Other veterinary samples that countries may consider include:

(i.) associated environment samples (water, effluent, surfaces) ; and
(ii.) animal feed (forage, feed).

3.3. 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 (WOAH, 2021a). It is important that only well-trained lab technicians in standard sampling procedures perform the sample collection, as per attached standard operating procedures (SOPs) listed in Annex 7 and attached as separate documents. Sterile techniques must be used for sample collection and samples need appropriate storage to avoid contamination.

3.3.1. Collection of samples at slaughter

3.3.1.1. Caecum from broilers/layer/free range chicken

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. 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 samples from the same batch is permissible, as necessary. If so, 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.

3.3.1.2. Chicken carcasses and meat swabs

The caecum and whole carcass must be from the same slaughter batch for the poultry. 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 skin from the neck and breast of the whole carcass collected are used for examination of *Salmonella* and *Campylobacter.*
In case of cattle, sheep, goats and camels, meat swabs should be included. These should match the faecal/intestinal contents sampled from the same animals.

3.3.1.3. Caecal content from pigs

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

3.3.1.4. Faecal/intestinal content from Cattle, Sheep, Goats and Camels

Faecal contents are taken from the colon or rectum after the 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 enough material for bacterial isolation is taken.

3.3.2. Collection of samples at the farm level

3.3.2.1. Nasal swabs from pigs

Sampling will be conducted at various pig farms in different regions of the country. Push the swab deep into the ventral passage of the nose and leave it in this position for three seconds; to avoid bleeding, do not injure the nasal conchae by applying too much pressure. Rotate the swab one-third around the central axis and leave it again for three seconds. Again, rotate the swab one third around the central axis and leave it for three seconds. Place the swab in the appropriate container. Two nasal swabs (from each nostril) to be collected (using swabs moistened by peptone water) from the same animal (pigs and cattle) and put in 2 ml of peptone water.

3.3.2.2. Milk from Cows, Camels, Sheep and Goats

Samples for culture should be collected immediately before milking to test for any zoonoses. To minimize contamination and maximize the chances of receiving useful information from the milk culturing process, adhere to the guidelines for the aseptic collection of clean milk samples. Hand swabs from the milkers should also be taken, to check for any possible contamination (e.g. for *Staphylococcus aureus).* Sampling will be conducted at various farms or milk collection centres in different regions of the country.

3.3.2.3. Boot swabs

In broiler and laying hen farms, faecal material should be collected to maximize the sensitivity 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 size of the poultry house and number of birds should determine the division of the floor area of the house into equal sectors for sampling. The number of steps walked by the staff per pair of boot swabs within the chosen sector will be determined by the size of the farm to ensure that all parts of the sector are sampled. Once the sampling has been 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, as per the SOP in Annex 7.

Preparation of boot swabs:

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

3.4. 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 zip lock envelope on the outside of the shipping container and should always accompany the samples to the laboratory. Microbiology laboratories should record the data of the sampling. Examples of sampling information are presented in Table 3.1 and Table 3.2. This information should be modified according to the planned data management strategy of the country.

Table 3.1 Examples of sample information collected from farm/sample submission form

Details	Information
Sender/sample collector details	
Name	
Contact details (mobile/email/telephone/fax numbers)	
Sample collection details	
Date and time of sampling	
Type of farm	
Location/geographical origin (GIS data where available)	

Details	Information
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	

Table 3.2 Examples of sample information collected from slaughterhouse/slaughter point

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. age, condition, health status, identification, sex)	

3.5. Packaging, transportation, and reception 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 analysed (WOAH, 2017). The samples must be always kept at 4–8 °C from the point of collection until processed (Table 3.3).

Table 3.3 Summary sample of types of packaging and transport requirements

Sample type	Transport time	Storage/ transportation temperature	Transport media	Maximum time before processing
Ceacal, faecal or intestinal content	6–8 hours 2 hours	4–8 ^{0}C Room temperature	Cary–Blair	72 hours
If Campylobacter spp. is suspected	High temperatures (>20 °C), low temperatures and fluctuations in temperature must be avoided.	4–8 ^{0}C	Amies	
Carcass or meat swabs	6–8 hours	4–8 ^{0}C		72 hours
Boot swabs	6–8 hours	4–8 ^{0}C		72 hours
Milk	6–8 hours	4–8 ^{0}C		
Nasal swabs	6–8 hours	4–8 ^{0}C	Stuarts	72 hours
Eggs		4–8 ^{0}C or room temperature		

3.5.1. Packaging of the samples

Samples should be placed in secure cool box containers for transport. Normally, well-packed samples should be placed in cool boxes together with frozen gel packs. The samples should never be placed directly on the frozen gel packs as this will freeze the samples which may kill bacteria. The following should be carefully noted:

(i.) all specimens should be packaged and transported in accordance with local and/or national regulations;
(ii.) procedures should minimize the risk of exposure for those engaged in transportation and should protect the environment and susceptible animal populations from potential exposures;
(iii.) specimens should always be packaged and transported to protect the integrity of the specimens, as well as to avoid cross-contaminating other specimens; and
(iv.) minimal requirements for transporting specimens follow the principle of triple packaging, which consists of three layers – a watertight and leakproof primary inner receptacle, durable, watertight, leakproof secondary packaging that will protect the primary packaging, and sturdy outer packaging that will protect the two layers against physical damage while in transit.

3.5.2. Sample transportation and storage

The samples should be transported to the laboratory immediately or within 6–8 hours after collection. In the case that 6 – 8 hours (or even less for some organisms such as *Campylobacter*) is not possible, particularly when dealing with remote areas far from laboratory use of transport media (Table 3.3) is recommended. 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). Where possible, a thermal data logger should be used when transporting the samples to the laboratory to ensure temperature monitoring. The use of transport media is also encouraged, especially where delays to processing can be foreseen.

3.5.1.2. Transport media

These are essentially solutions of buffers with carbohydrates, peptones and other nutrients (excluding growth factors), designed to preserve the viability of bacteria during transport without allowing them to multiply. Antibiotics and other substances like glycerol may be added. Transport media include:

(i.) Cary-Blair medium for stool samples;
(ii.) Amies medium with charcoal for samples to isolate *Campylobacter;*
(iii.) Stuart's medium for transporting wound and skin swabs that may contain fastidious organisms;
(iv.) Sach's buffered glycerol saline for bacillary dysentery stool samples; and
(v.) Anaerobic transport medium, such as a Thioglycolate broth.

a. Caecal or faecal samples

These samples should be transported immediately and arrive at the laboratory within six to eight hours after collection. The analysis should be performed immediately. If this cannot be managed, the samples should be stored at 4 °C to 8 °C and analysed no later than 72 hours after sampling.

b. Carcass or meat samples

The sample should be kept at 4 °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 six to eight 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 at the laboratory no more than three days after sampling, but this is not ideal. The sample should be stored at 4 °C to 8 °C after arrival. The analysis should be performed within 24 hours from the time of arrival in the laboratory.

c. Boot swabs

Boot swab samples should be transported within the same day of sampling and arrive at the laboratory within six to eight hours after collection. At the laboratory, samples should be kept refrigerated (4 °C to 8 °C) until examination. The laboratory analyses should be carried out within 24 hours after receipt or 72 hours after collection (EU Health, 2005).

d. Milk samples

These samples should be transported immediately and arrive at the laboratory within six to eight hours after collection. The analysis should be performed immediately. If this cannot be managed, the samples should be stored at 4 °C to 8 °C until analysed, which should be no later than 72 hours after sampling.

e. Nasal swabs

These samples should be transported immediately and arrive at the laboratory within six to eight hours after collection. The analysis should be performed immediately. If this cannot be managed, the samples should be stored at 4 °C to 8 °C until analysed, which should be no later than 72 hours after sampling.

f. Eggs

Whole eggs shall be collected from the poultry house in proportion to the average number of eggs laid and as calculated by the epidemiology team. From each egg, the following shall be sampled for analysis: external shell membrane, the shell, internal shell membrane, the egg white and the vitelline membrane separating the white from the yolk.

CAMPYLOBACTER SPP.

Campylobacters spp. are sensitive to environmental conditions, including dehydration, atmospheric oxygen, sunlight and elevated temperature. Transport to the laboratory and subsequent processing should 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. There is no recommendation on the ideal temperature for transportation, however, 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 to 8 °C is advised (WOAH, 2017).

3.5.3. Sample submission/reception in the laboratory

Sample reception is an important pre-analytical procedure that, if not well-organized, can compromise the integrity of the entire workflow. A well-documented procedure on sample reception and rejection criteria will enhance and guarantee the traceability of samples (as per attached SOPs, Annex 7).
A good sample reception SOP should have:
(i.) criteria for accepting or rejecting the sample;
(ii.) method for logging the samples in the physical or electronic register;
(iii.) a method for assigning laboratory identifications; and
(iv.) a plan for handling urgent samples.
Generally, samples may be rejected if:
(i.) samples are unlabelled or improperly labelled;
(ii.) samples with labels that do not match details on the request form;
(iii.) request forms have insufficient information or are incomplete;
(iv.) information on tests requested is missing;
(v.) specimen has been collected in the wrong container;
(vi.) samples are leaking;
(vii.) request forms do not accompany specimens and vice versa;
(viii.) test requested is not available;
(ix.) test order has been cancelled or wrong order provided;
(x.) specimen delayed in transit; or
(xi.) there is insufficient volume to test.

Chapter 4 - Laboratory methods

Sample processing is an important part of specimen management. Properly processed specimens ensure the accuracy and thus the reliability of the laboratory results. Safety practices need to be assessed and reviewed as necessary as pertaining to all laboratory activities to include the pre-analytical (specimen collection and processing), analytical (specimen testing) and post-analytical (specimen disposal/waste management and reporting) processes.
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 and the results are interpreted. See attached SOPs, Annex 7.

4.1. Processing faeces/caecal contents

When processing caecal contents in the laboratory for isolating and identifying target bacteria, 28 g of samples are needed to perform the analyses for *Salmonella* spp., *Campylobacter spp., E. coli* and *Enterococcus* spp. The 28 g portion of the sample is transferred to 252 ml of buffered peptone water (BPW) or campylobacter enrichment broth (CEB) at room temperature. The mixture is then vortexed for one minute. This initial suspension shall be used as identified in Table 4.1.

Table 4.1 Distribution of initial suspension (28 g) of faecal sample

Volume	Utilization	Related sections
250 ml (~25 g)	For the detection of *Salmonella* spp.	Section 4.6.1
10 ml (~1 g)[a]	For the detection of *E. coli*	Section 4.6.2
10 ml (~1 g)[a]	For the detection of *Campylobacter* spp.	Section 4.6.3
10 ml (~1 g)	For the detection of *Enterococcus* spp.	Section 4.6.4
10 ml (~1 g)	For the detection of *Staphylococcus* aureus	Section 4.6.5

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

It should be noted that the objective is not to determine 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).

4.2. Processing meat (broiler carcasses, red meat) in the laboratory

Broiler carcass: Using aseptic techniques, the neck skin (if present) and the skin from one side of a broiler 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 bag. Any fat should be avoided (EFSA, 2010).

Meat (from cattle, shoats, goats, sheep and camels): The meat sample or meat swabs arrive at the laboratory in sealed sample/sponge bags. Based on (Horwitz, W., 1975; U.S. Meat Animal Research Center (2003); Dolinsky (2017) and Luangtongkum *et al.* (2007).

(i.) The sponge bags are massaged, and 2.5 ml aliquot removed before the enrichment media is added. The aliquot is used to determine the presence of aerobic bacteria, Enterobacteriaceae, *E. coli* 0157:H7, and Salmonellae (as per the SOPs, Annex 7).

(ii.) 80 ml of tryptic soy broth (TSB) is added to the sample bags.
(iii.) All sample bags are homogenized by hand massaging then they are incubated for two hours at 25 °C then for six hours at 42 °C (and stored at 4 °C overnight).
(iv.) One ml of each enrichment is plated on sorbitol MacConkey agar (SMAC) supplemented with 0.05 mg/L cefixime, 2.5 mg/L potassium tellurite, and a chromogenic media.
(v.) The plates are incubated for 16 to 24 hours at 37 °C. Up to five suspect colonies should be picked and tested by latex agglutination.
(vi.) Salmonella present in the samples is detected by swabbing the enrichment onto (i) Hektoen enteric agar containing novobiocin (5 mg/l) and (ii) Brilliant Green medium with Sulfadiazine, incubating at 37 °C for 18 to 20 hours.
(vii.) Suspect colonies are picked for confirmation by polymerase chain reaction (PCR) for the Salmonella-specific gene *invA*. (1,2).

4.3. Processing boot/sock swabs in the laboratory for bacterial pathogen isolation

If possible, boot swabs should be gathered at the 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 it 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 or CEB. The bag/jar is gently swirled and then placed in the incubator at 37 °C overnight.

4.4. Processing milk samples

For each milk sample, there will be a tenfold serial dilution. Up to 10^6 dilutions will be prepared using BPW. From each dilution, 1 ml will be cultured in appropriate *Staphylococcus* supporting media. *S. aureus* produces the yellow pigment staphyloxanthin and characteristic gold-coloured colonies are formed on all rich media including tryptic soy agar (TSA) at 37 °C, brain heart infusion agar and Luria Bertani agar. The inoculated agar plates will be incubated at 37 °C for 24 to 48 hours. Colony morphology and different primary and secondary biochemical tests will be used to identify bacteria to the species level.

4.5. Processing nasal swabs

The samples will be inoculated in TSB with 10 percent sodium chloride salt (TSB-S) and incubated at 37 °C for 24 hours. The samples which show turbidity in TSB-S broth will be streaked on mannitol salt agar with 6 mg/L oxacillin (O-MS agar) and incubated for 24 hours at 37 °C. Bacterial growth with mannitol fermentation will be observed for the presence of round, typical golden, yellow or pale colour colonies of oxacillin-resistant staphylococci. The presumptive isolates will be further confirmed by Gram's staining and catalase test.

4.6. Bacteria isolation methods

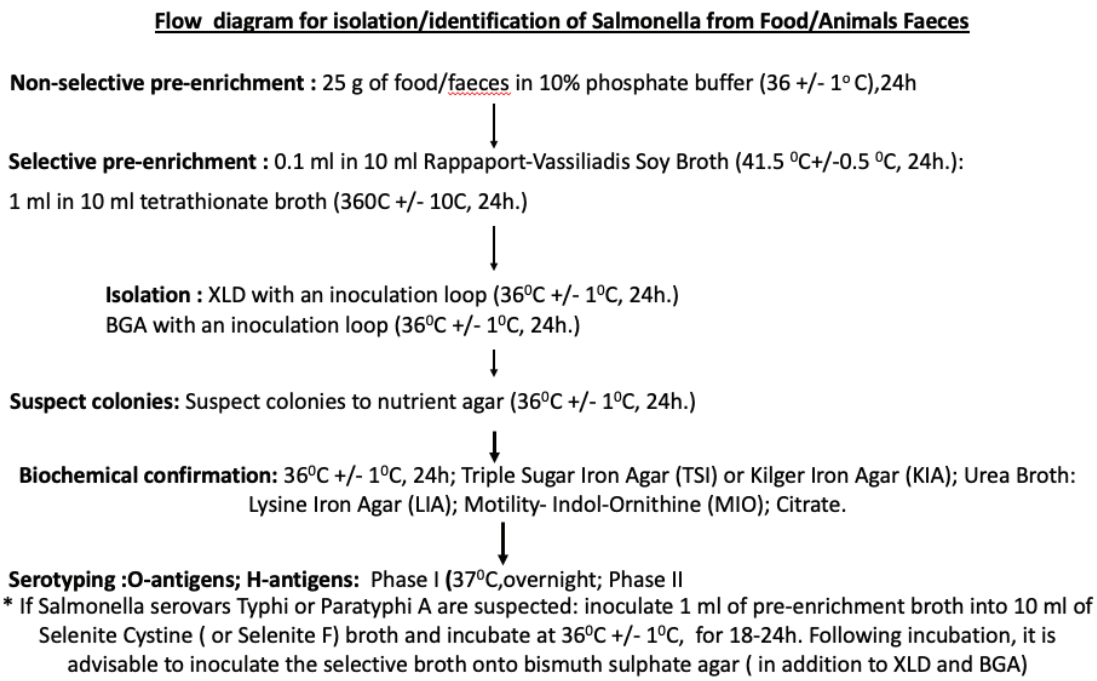
For these guidelines, both zoonotic and commensal species of bacteria will be isolated and identified using microbiological techniques such as the Gram stain reaction, morphology and biochemical reactions. Bacterial isolation, purification and identification are important components in AMR surveillance. Pure culture is essential in the study of the morphology, physiology, biochemical characteristics and susceptibility to antimicrobial agents of a particular bacterial strain. Pure cultures

will be obtained by a streak plate method where a loopful of the inoculum is placed near the periphery of the plate with agar medium and spread or streaked on the upper portion of the plate with parallel overlapping strokes, detailed below and as per the SOPs in Annex 7.

4.6.1. Isolation and serotyping of *Salmonella* spp.

There are numerous methods for isolating and detecting *Salmonella* used worldwide, but the increasing application of external quality assurance programmes has led to greater use of international standard (ISO) 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 isolating Salmonella from food, feedstuffs, faecal and environmental samples as outlined in WOAH (2017) may be followed as shown in Figure 4.1.

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



Source: WOAH. 2022. *WOAH Terrestrial Manual* 2022. Chapter 3.10.7. Salmonellosis. Paris, World Organisation for Animal Health.

At least three isolates from each positive sample should be typed for their serotypes following the Kaufmann–White scheme (Popoff and LeMinor, 1992) or *Microbiology of the food chain – Horizontal method for the detection, enumeration and serotyping of Salmonella spp. Part 3: Guidelines for serotyping of Salmonella* spp., www.iso.org ISO/TR 6579–3:2014.

4.6.2. Isolation and identification of *Escherichia coli*

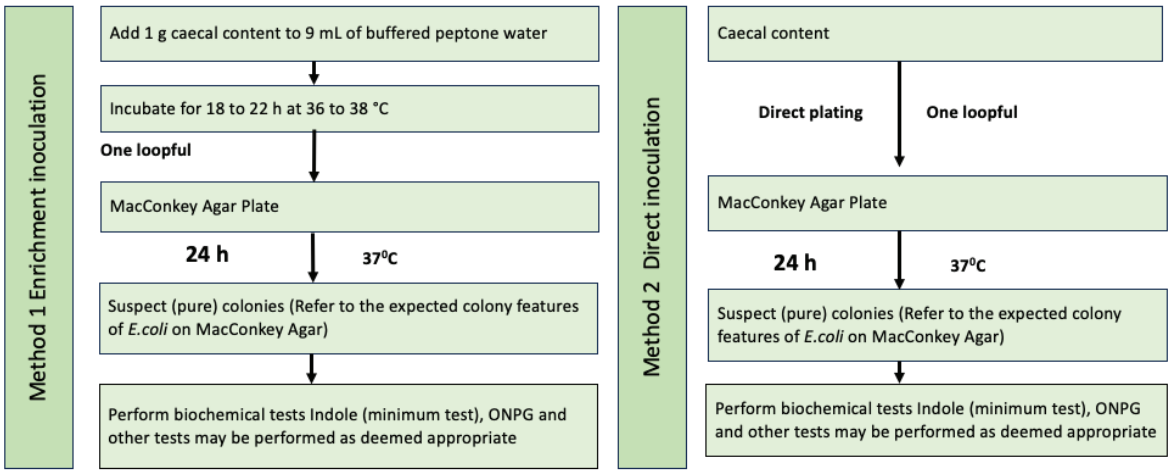
Method 1: Enrichment inoculation

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 to 22 hours. 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 BAM4, available at 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.

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 the species level. The minimum requirement is to test for indole production for verification of the species. Theortho-nitrophenyl-β-D-galactopyranoside test (ONPG) can be additionally used to verify presumed *E. coli* isolates.
The steps taken to perform enrichment inoculation and direct inoculation are shown in Figure 4.2.

Figure 4.2 Basic methods for detecting and identifying *E. coli*



Notes: Method 1, in case of swabs or delayed processing. Method 2, direct/immediate processing of faecal or caecal samples.

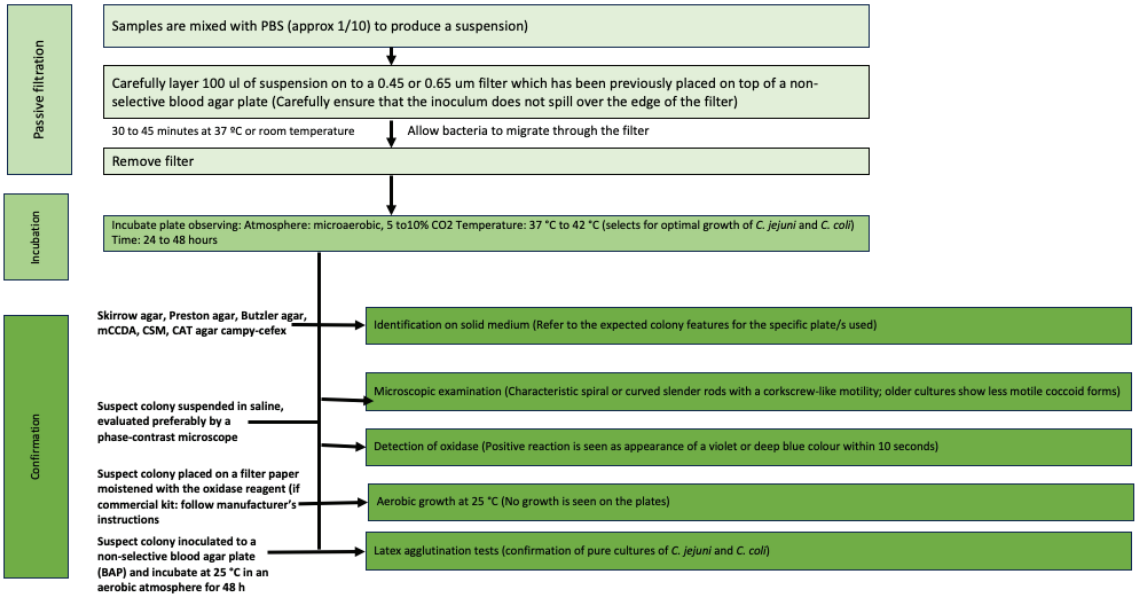
Source: Detection and Typing Strategies for Pathogenic Escherichia coli (2015). Lucia Rivas, Glen E. Mellor, Kari Gobius, Narelle Fegan. Book, in SpringerBriefs in Food, Health, and Nutrition.

4.6.3. 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 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 Microbiology of food and animal feeding stuffs – *Horizontal method for detection and enumeration of Campylobacter* spp.

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 WOAH (2018) may be followed.
Samples can be plated on selective medium (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. PCR) or matrix-assisted laser desorption/ionization time of flight (MALDI-TOF) mass spectrometry. The methodology and specific primers set of each species is included in the SOPs in Annex 7.

Figure 4.3 Isolation and confirmation of *Campylobacter spp.*



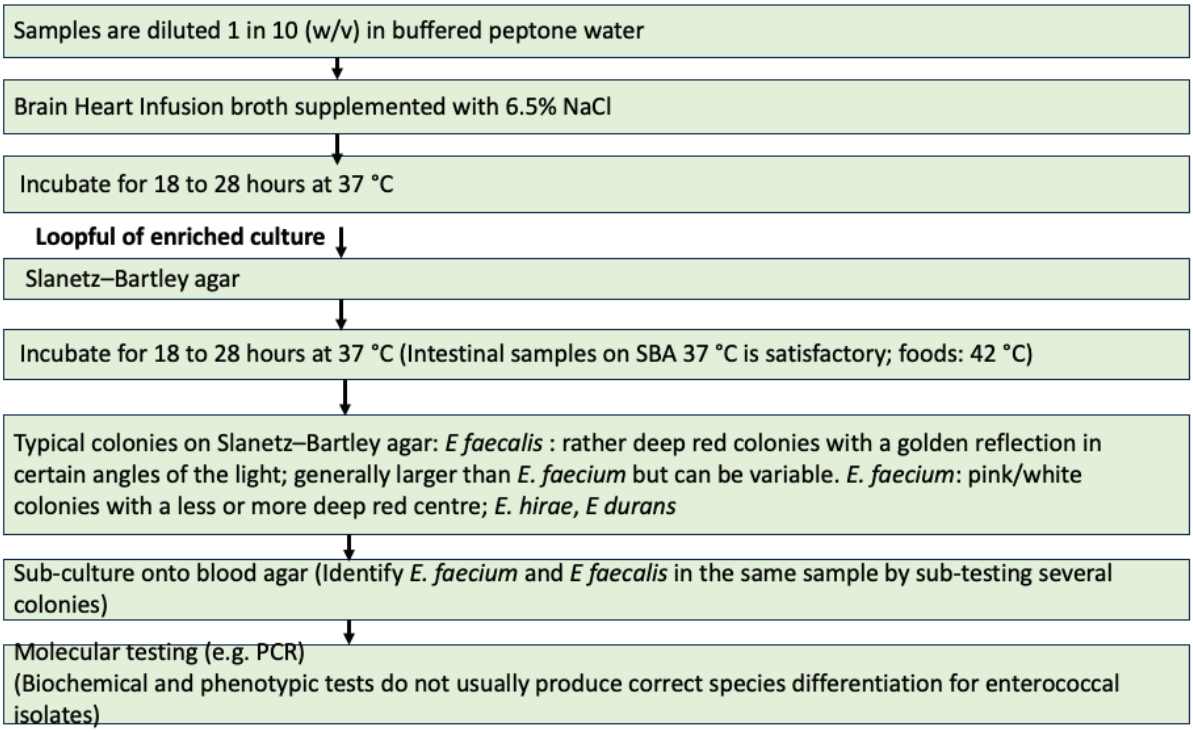
Source: NATSOS *et al*. 2019. Atsos, G., Mouttotou, N., Ahmad, S., Kamran, Z., Ioannidis, A., & Koutoulis, K. (2019).
The genus *Campylobacter*: detection and isolation methods, species identification & typing techniques. Journal of the Hellenic Veterinary Medical Society, 70(1), 1327-1338. doi. http://dx.doi.org/10.12681/jhvms.20337 [accessed Sep 04 2022].

Notes: These are suggested media; however, each Member Country could decide on which media to use based on preference and available resources.

4.6.4 Isolation and identification of *Enterococcus* spp.

Different methods can be used for primary isolation of *Enterococcus* spp. Several different species of *Enterococcus* can be found as commensals in the gastrointestinal 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. Detecting and identifying *Enterococcus* spp.



Source: EFSA. 2008. Report from the Task Force on Zoonoses Data Collection including guidance for harmonized monitoring and reporting of antimicrobial resistance in commensal *Escherichia coli* and *Enterococcus* spp. from food animals. The EFSA Journal, 141:141-144.

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, and PCR procedures can be used based on those used previously by Dutka-Malen, Evers and Courvali (1995).

4.6.5. Isolation and identification of *Staphylococcus Aureus*

The growth and survival of *S. aureus* relies on environmental factors, such as temperature, water activity and pH, presence of oxygen, and composition of the sample. The morphology of suspected colonies is determined by shape, size, colour and microscopic examination including Gram stain. The suspected colonies are confirmed by biochemical reactions including the Coagulase test, Catalase test, Oxidase, DNase test and beta haemolysis test on blood agar, as per attached SOP in Annex 7.

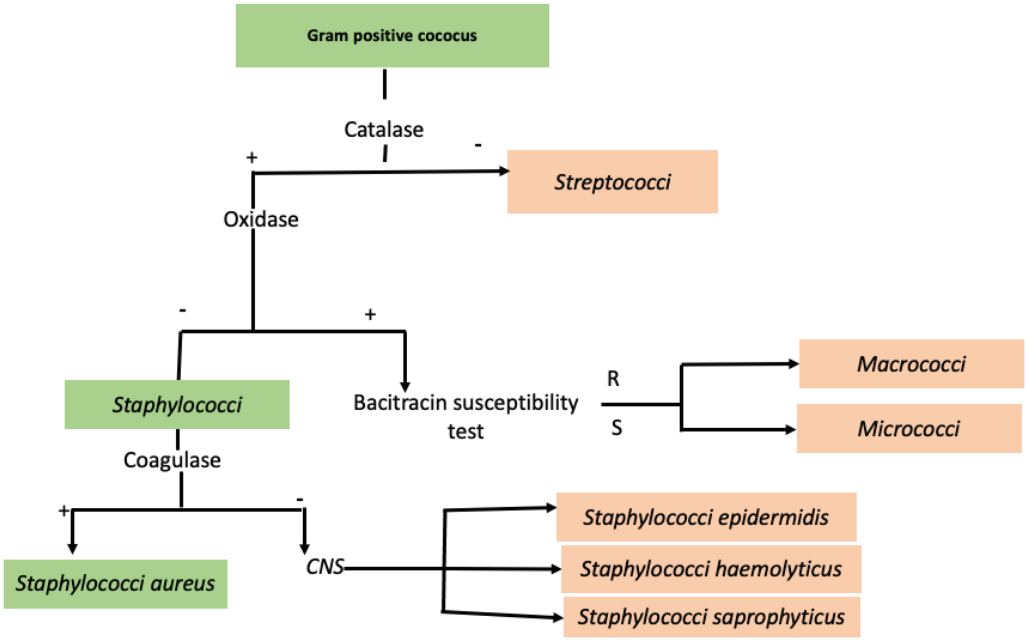
From suspected colonies, proceed to staphaurex test to confirm and differentiate *S. aureus* from other *Staphylococcus* species.

Staphylococcus aureus latex agglutination test

The *Staphylococcus aureus* latex reagent consists of polystyrene latex particles, which have been coated with fibrinogen and IgG. When mixed on a slide with a suspension of *S. aureus* organism, the reaction of clumping factor with the fibrinogen, and/or of protein A with IgG causes rapid, strong agglutination of the latex particles, refer to SOP in Annex 7.

Detection and characterization of *Staphylococcus Aureus*

Figure 4.5 Identifying *Staphylococcus aureus* from other *Staphylococcus* spp.



Notes: The volumes are indicated in the SOPs. This flow chart provides a general elimination method to arrive at *S. aureus*.
Source: Medical Microbiology. 4th edition. Baron S, editor. Chapter 12 Staphylococcus Galveston (TX): University of Texas Medical Branch at Galveston; 1996.

4.7. Antimicrobial susceptibility testing

There are various methods for in vitro AST (disc diffusion, E-test, sensi-titre, agar dilution, broth microdilution and broth macrodilution).

Table 4.2. Merits and demerits of various antimicrobial susceptibility methods

AST method	Merits	Demerits
Disc diffusion: Standard concentration of an organism is plated onto Mueller–Hinton agar. Afterwards, paper discs containing fixed concentrations of antibiotics are placed onto the surface of the media.	1. It is cheap, flexible and allows visibility of growth, correct inoculum, mixed cultures and other abnormalities. 2. There is the possibility of executing direct susceptibility testing.	1. Results may be unexpected or borderline.
E-test: Non-porous plastic reagent strip with a predefined gradient of antibiotic, covering a continuous concentration range is applied to the surface of an agar plate inoculated with the test strain.	1. It is flexible in the number and kind of antimicrobial agents that can be tested. 2. It is easy to set up individual tests. 3. There is accuracy in obtaining the MICs.	1. Only one drug can be tested at a time.
Sensi-titre minimum inhibitory concentration (MIC) susceptibility system is for in vitro susceptibility testing. It utilizes broth micro dilution method, providing qualitative (S,I,R) and quantitative MIC results.	Results can be read automatically.	1. It is time bound and 24 hour manual reads can't be performed after 24.5 hours of incubation. 2. If there is not enough organism to make 0.5 McFarland, it can show false susceptibility and vice versa if over 0.5. 3. If the wrong amount (dilution) is added to Mueller– Hinton broth, the results would be invalid. 4. If inoculated panel sits too long there can be false susceptibility. 5. Wells dry out if not covered properly.
Agar dilution: Involves incorporation of different concentrations of the antimicrobial substance into an agar medium then application of a standardized number of cells to the surface of the agar plate.	1. Accurately determines MICs. 2. It is possible to test many organisms against a series of dilutions of a single antimicrobial at the same time.	1.It is labour-intensive and expensive.
Broth microdilution: Multiple microtiter plates are filled with a broth composed of supplements. Varying concentrations of the antibiotics and the bacteria to be tested are then added to the plate. The plate is then placed into a non-CO2 incubator and incubated at 35 °C for 16 to 24 hours.	1. Ability to test the susceptibility of microorganisms to multiple antibiotics at once; 2. Is highly accurate.	1. It is a time-consuming; 2. Not cost-efficient; 3. It requires the availability of the antimicrobial agents to be tested as pure substance; 4. No automation is possible.

Broth macrodilution: 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		
Molecular methods	1. It is highly automated; hence it can process large volumes in one run 2. Very accurate.	1. Initial capital cost for setup and human resource training may be costly.

For these guidelines, disc diffusion and E-test will be used. The tests must be performed in accordance with internationally accepted procedures such as those published by the Clinical and Laboratory Standards Institute (CLSI) VET01. 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.

The selection of an AST methodology may be influenced by ease of performance, flexibility, adaptability to automated or semi-automated systems, cost, reproducibility, reliability, accuracy, organisms and the antimicrobials of interest in that particular WOAH member and availability of suitable validation data for the range of organisms to be susceptibility tested.

4.7.1. Disk diffusion (Kirby-Bauer)

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 (WOAH, 2012). This is the most widely available method for performance of AST in the Eastern Africa region. It is technically simpler to perform, less expensive and useful for guiding treatment by providing categorical information (susceptible, intermediate, or resistant) (EFSA, 2007; 2008; 2019). However, it should be noted that the disk diffusion method 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 diametres correlate inversely with MIC breakpoints, regression line analysis should not be used to extrapolate MIC values from measurements of zones of inhibition. Although this may be mathematically correct in many cases, 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.

4.7.2. E-test

An E-test determines the MIC of an organism to a drug. E-tests utilize a rectangular plastic strip that has been impregnated with a stable gradient of 15 concentrations of an antimicrobial agent. The method is based on the diffusion of a continuous concentration gradient of an antimicrobial agent from the plastic strip into an agar medium. When the E-test strip is applied to an inoculated agar plate, there is an immediate release of the drug and an antimicrobial concentration gradient is

established in an agar medium. After overnight incubation, the tests are read by viewing the strips from the top of the plate, and a symmetrical inhibition ellipse is produced. The intersection of the lower part of the ellipse-shaped growth inhibition area with the test strip indicates the MIC value.

4.7.3. Molecular methods

The advantage of molecular methods, in addition to phenotypic methods, is their speed and accuracy in detecting the underlying genetic determinants of AMR. Some examples of methods include PCR, deoxyribonucleic acid (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 not yet ready for this.

Additionally, measures such as the epidemiological cut-off value (ECOFF), which is valuable in AMR surveillance, cannot be measured using these methods. However, molecular techniques can be used to identify some bacterial species (e.g. *Campylobacter, Enterococcus*).

Recommendations regarding choice of susceptibility testing methods: For the 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 the European Committee on Antimicrobial Susceptibility Testing (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 the performance and reproducibility of the test system (Table 4.4).

4.8. Interpretation of test results

Currently, two 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.

4.8.1. Clinical breakpoints

Clinical breakpoints (CBPs) are numerical 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, 2019).

4.8.2. Epidemiological cut-off values

Epidemiological cut-off values (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. The European Committee on Antimicrobial Susceptibility Testing (EUCAST) introduced the term microbiological resistance and presents ECOFFs for antimicrobials against a wide range of bacteria (EFSA, 2012a; 2019). Table 4.3 presents a comparison of CBPs and ECOFFs and more detailed information on CBPs and ECOFFs can be found in (CLSI, 2011).

Table 4.3 Comparison of clinical breakpoints and epidemiological cut-off values

	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 antimicrobial 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

4.9. Interpretive criteria and antimicrobial resistance 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 4.4A, 4.4B and 4.4C.

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 be compared with care. There is still a considerable need to harmonize the process for agreeing on 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. 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 (CLSI (2018) and CLSI (2019), it is important to highlight that CLSI standards are best viewed holistically in their entirety.

4.10. Quality control in antimicrobial susceptibility testing

The quality control 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 the 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 the 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.

4.11. 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 4.4A, Table 4.4B, Table4.4C and Table 4.4D together with interpretive criteria for disk susceptibility testing. Additional antimicrobial agents can be included, depending on country needs and preferences. The values included in the tables are based on the 2020 CLSI guideline and for the disc diffusion method.

These values may vary depending on the edition of the guidelines in effect at the time of use. Therefore, countries are encouraged to refer to the latest version of the CLSI guidelines while interpreting the zones of inhibition or MIC values. The list of antimicrobials also includes the proposed defined panel of antimicrobials for monitoring and evaluation of the GAP in the core indicator for *E. coli*.[1]

[1] https://cdn.who.int/media/docs/default-source/antimicrobial-resistance/amr-spc-npm/m-e-framework-annex3-methodology-sheets.pdf?sfvrsn=55744418_7

Table 4.4A Interpretative criteria for *Salmonella* spp. and *E. coli* antimicrobial susceptibility testing using a disc diffusion technique

Relevant antimicrobials [a]	Disc content	Interpretation (diameter mm)			
		S	I	R	Classification and prioritization[e]
Ampicillin	10µg	≥17	14–16	≤13	Highest priority critically important antimicrobials
Cefotaximea[b]	30µg	≥26	23–25	≤22	
Ceftazidime[b]	30µg	≥21	18–20	≤17	
Nalidixic acid	30 µg	≥19	14–18	≤13	
Ciprofloxacin	5 µg (for other enterobacterial)	≥26	22–25	≤21	
	5 µg (for Salmonella spp.)	≥31	21–30	≤20	
Colistinc		NA	>NA	NA	
Relevant antimicrobials [a]	**Disc content**	**Interpretation (diameter mm)**			
		S	**I**	**R**	**Classification and prioritization[e]**
Gentamicin	10 µg	≥15	13–14	≤12	High priority critically important antimicrobials
Streptomycin	10 µg	≥15	12–14	≤11	
Meropenem[d]	10 µg	≥23	20–22	≤19	
Chloramphenicol	30 µg	≥18	13–17	≤12	Highly important antimicrobials
Sulphamethoxazole	23.75 µg	≥17	13-16	≤12	
Trimethoprim	1.25 µg	≥16	11–15	≤10	
Tetracycline	30 µg	≥15	12–14	≤11	

Source: The European Committee on Antimicrobial Susceptibility Testing. Breakpoint tables for interpretation of MICs and zone diameters. Version 13.1, 2023. http://www.eucast.org

Notes: S = Sensitive, I = Intermediate, R = Resistant; [a]2020 CLSI guideline; [b]Breakpoints are based on a dosage regimen of 1 g administered every eight hours for cefotaxime and ceftazidime; [c]Several species are intrinsically resistant to the lipopeptides (colistin and polymyxin); [d]Breakpoints are based on a dosage regiment of 1 g administered every eight hours.

Table 4.4B Antimicrobial panel and interpretive criteria for *Campylobacter jejuni* and *C. coli*

Relevant antimicrobial group[a]	Disc content	Interpretation (diameter mm)			
		S	I	R	Classification and prioritization
Ciprofloxacin[b]	5µg	≥21	16–20	≤15	Highest priority critically important antimicrobials
Nalidixic acid	30 µg	≥19	14–18	≤13	
Erythromycin	15 µg	≥23	14–22	≤13	
Gentamicin	10 µg	≥15	13–14	≤12	High priority critically important antimicrobials
Streptomycin	10 µg	≥21	16–20	≤15	
Tetracycline	30 µg	≥19	15–18	≤13	Highly important antimicrobials

Source: The European Committee on Antimicrobial Susceptibility Testing. Breakpoint tables for interpretation of MICs and zone diameters. Version 13.1, 2023. http://www.eucast.org

Notes: S = Sensitive, I = Intermediate, R = Resistant; a 2020 CSLI guideline; b susceptibility to fluoroquinolones give no zone inhibition with a nalidixic acid 30 µg disc.

Table 4.4C Antimicrobial panel and interpretive criteria for *Enterococcus* spp. using disc diffusion technique

Relevant antimicrobial group[a]	Disc content	S	I	R	Classification and prioritization
Erythromycin	15 µg	≥23	14–22	≤13	Highest priority critically important antimicrobials
Teicoplanin	30µg	≥14	11-13	≤10	
Vancomycin	30 µg	≥17	15-16	≤14	
Ampicillin	10 µg	≥17	-	≤16	Highly important antimicrobials
Rifampicin	5 µg	20	17–19	≤16	
Ciprofloxacin	5 µg	21	16–20	≤15	
Linezolid	30 µg	≥23	21-22	≤20	
Quinupristin/ dalfopristin	15 µg	≥19	16-18	≤15	Highly important antimicrobials
Chloramphenicol	30 µg	≥18	13-17	≤12	
Tetracycline	30 µg	≥19	15-18	≤14	

Source: The European Committee on Antimicrobial Susceptibility Testing. Breakpoint tables for interpretation of MICs and zone diameters. Version 13.1, 2023. http://www.eucast.org

Notes: S = Sensitive, I = Intermediate, R = Resistant; a 2020 CSLI guideline.

Table 4.4D Antimicrobial panel and interpretive criteria for *Staphylococcus aureus* using disc diffusion technique

Relevant antimicrobial group[a]	Disc content	S	I	R	Classification and prioritization
Cefoxitin (Oxacillin) *S. aureus*		-	-	-	Highest priority critically important antimicrobials
Other *Staphylococcus* spp.	30µg	≥25	-	≤24	
Erythromycin	15 µg	≥23	14-22	≤13	
Vancomycin		-	-		
Trimethoprim/ sulphamethoxazole	1.25/23. 75 µg	≥16	11-15	≤10	High priority critically important antimicrobials
Gentamicin	10 µg	≥15	13-14	≤12	
Ciprofloxacin	5 µg	≥21	16-20	≤15	
Clindamycin	2 µg	≥21	15-20	≤14	
Linezolid	30 µg	≥21	-	≤20	
Quinupristin/ dalfopristin	15 µg	≥19	16-18	≤15	Highly important antimicrobials
Chloramphenicol	30 µg	≥18	13-17	≤12	
Tetracycline	30 µg	≥19	15-18	≤14	

Source: The European Committee on Antimicrobial Susceptibility Testing. Breakpoint tables for interpretation of MICs and zone diameters. Version 13.1, 2023. http://www.eucast.org
Notes: S = Sensitive, I = Intermediate, R = Resistant; a 2020 CSLI guideline.

Given that this particular surveillance of the human–animal interface is for protecting public health, the panel was anchored on the most relevant antimicrobials to humans as per WHO classification (WHO, 2016; 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. Additional references for Sections 4.1 to 4.12 include Horwitz (1975), US Meat Animal Research Center (2003), Dolinsky (2017), Luangtongkum *et al.* (2007).

4.12. Complementary antimicrobial susceptibility testing

Detecting extended-spectrum β-lactamases (ESBL)-producing and carbapenem-resistant bacteria in animal populations is very important. Phenotypic testing for ESBL production and carbapenem resistance in *Salmonella* and *E. coli* should be performed when possible (see Figure 4.5). Additional phenotypic testing (ESBL e-tests, combination discs, double-disc synergy methods on Mueller–Hinton (MH) agar and cloxacillin-containing MH agar, and the Cica–Beta test)– See Annex 7 for List of Specific standard operating procedures.

ESBL, AmpC or ESBL+AmpC phenotypes should be differentiated among extended-spectrum cephalosporin 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 producing *Salmonella* spp. and *E. coli*

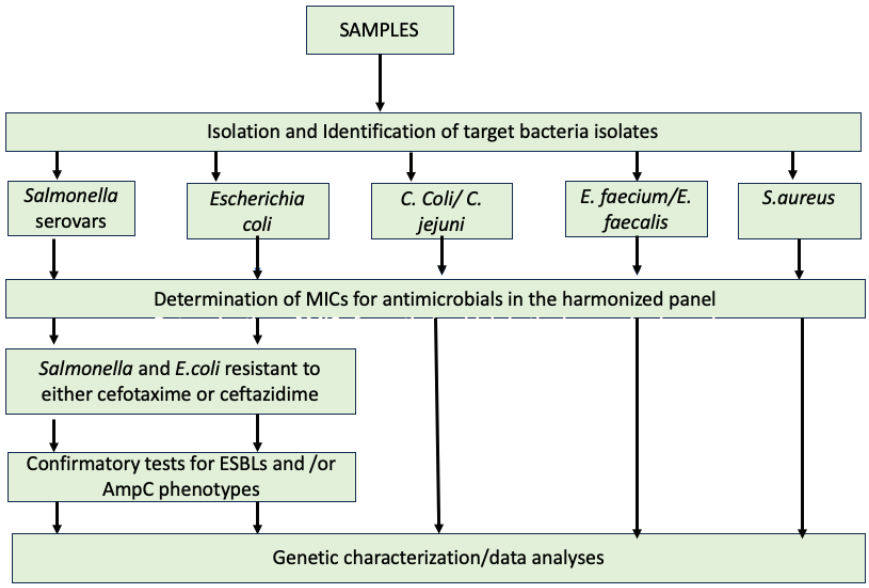
Cefotaxime and ceftazidime are included in the harmonized panel for routine monitoring. *Salmonella* or *E. coli* isolates that are resistant to either cefotaxime or ceftazidime should be further

confirmed for ESBL-production. The phenotypic confirmatory test requires the 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 disc diffusion or standard broth dilution as described in CLSI (2019).

(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 providing the best compromise between sensitivity and specificity in terms of detecting the majority of carbapenemases (EFSA, 2012b). 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 retested to confirm such resistance, which is likely to be rare in veterinary and food isolates. It is noted that detecting results of carbapenemase-producing Enterobacteriaceae should be reported quantitatively and not as interpreted value, as carbapenemase-producing Enterobacteriaceae often have MIC-values below the CBP. Screening and confirmatory test for CRE can be performed according to CLSI (2019).

Figure 4.6. Stepwise detection of antimicrobial susceptibility for *Salmonella, E. coli, Campylobacter, Enterococcus* and *S. aureus* isolates



4.13. Storage of the isolates

The bacterial isolates should be permanently preserved. The methods of storage must ensure viability and 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 used successfully for preserving microorganisms, but among these, cryopreservation and lyophilization are highly utilized for culture collection and industry.

4.13.1. *Cryopreservation*

Cryopreservation refers to the preservation of biological materials at cryogenic temperatures, generally at -80 °C or -196 °C. A 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. Using a 20 percent glycerol stock is recommended 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 ATCC (2015).

4.13.2 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: i) pre-freezing the product to form a frozen structure; ii) primary drying to remove most water; and iii) secondary drying to remove bound water. For more details, please refer to ATCC (2015) or Nata (1992).

4.13.3. Other methods

Methods that have been used successfully to preserve microorganisms include repeated subculturing, preserving on agar beads, overlaying slant-grown cultures, using silica gel and other sterile supports.

4.13.4. Additional notes on storing isolates

Apart from the brief review above of some of the common methods used to store isolated bacteria, 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 to be optimized 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 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 a preserved culture should be noted, as well as keeping track of the frequency that a stored vial has been used.

Chapter 5 - Data management and reporting

While acknowledging that there are different systems that have been developed and countries are using them, it is necessary to harmonize the data management system to make it easier to compare findings. Whichever system a country chooses to adopt should fit seamlessly into the microbiology laboratory database software WHONET, to enable data sharing. Database for animal and plant production subsectors are currently being developed. It is recommended that the Eastern African countries develop and implement a harmonized AMR database management system in the laboratories.

5.1. Data recording and storage

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

5.1.1. Recording and storing antimicrobial resistance data

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

(i). 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.

(ii). 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; or
b. as a diametre zone of inhibition in millimetres.

(iii). 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.

(iv). 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 type 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

A regional template (See Annex 6) is recommended as a convenient tool for simple AMR data entry and storage. The countries can use it to strategically collate AMR data generated in a harmonized approach. Developed by FAO, in Asia, the 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).

Although this template presents the opportunity to harmonize formats for ease of communication and possibly collating future 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. Each country should develop a data dictionary as an annex to its database. The administrative units should also be modified as appropriate to each country.

Table 5.1 Data definition matrix from the regional antimicrobial resistance 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]	Kenya
Subnational admin level 1 e.g. county in Kenya	Name of admin level 1 (if applicable)	Text	No limit	User defined[a]	Nairobi
Subnational admin level 2 (e.g. sub county in Kenya)	Name of admin level 2 (if applicable)	Text	No limit	User defined[a]	Westlands
Subnational admin level 3 (e.g. ward in Kenya)	Name of admin level 2 (if applicable)	Text	No limit	User defined[a]	Kangemi
Subnational admin level 4	Name of admin level 2 (if applicable)	Text	No limit	User defined[a]	N/A
Village	Name of village (if applicable)	Text	No limit	User defined[a]	Kangemi
Origin:	Describes the source of the specimen				
Farm number	Code for the farm (if applicable)	Text/ number	10 characters	User defined[a]	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
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]	Kenya
Subnational admin level 1 e.g. county in Kenya	Name of admin level 1 (if applicable)	Text	No limit	User defined[a]	Nairobi
Subnational admin level 2 (e.g. sub county in Kenya)	Name of admin level 2 (if applicable)	Text	No limit	User defined[a]	Westlands
Subnational admin level 3 (e.g. ward in Kenya)	Name of admin level 2 (if applicable)	Text	No limit	User defined[a]	Kangemi
Subnational admin level 4	Name of admin level 2 (if applicable)	Text	No limit	User defined[a]	N/A
Village	Name of village (if applicable)	Text	No limit	User defined[a]	Kangemi
Origin:	**Describes the source of the specimen**				
Farm number	Code for the farm (if applicable)	Text/ number	10 characters	User defined[a]	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-001t

Notes: aUser defined = countries should define naming/coding at the outset; bThere is a long list of options in the WHONET platform. If not using WHONET, then should be user defined.

The regional AMR data collection template (see Annex 6) also contains the list of antibiotics based on the harmonized panel of antimicrobials as listed in Section 4.3.4 for all target bacteria (*Escherichia coli, Salmonella* spp., *Staphylococcus aureus, 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 List of antibiotics included in the regional antimicrobial resistance data collection template

Code	Antibiotic	Code	Antibiotic	Code	Antibiotic	Code	Antibiotic
AMP	Ampicillin	CIP	Ciprofloxacin	MEM	Meropenem	TEC	Teicopžlanin
AZM	Azithromycin	COL	Colistin	QDA	Quinupristin/ Dalfopristin	TCY	Tetracycline
CAZ	Ceftazidime	GEN	Gentamicin	STR	Streptomycin	TMP	Trimethoprim
CHL	Chloramphenicol	LNZ	Linezolid	SMX	Sulfamethoxazole	VAN	Vancomycin

Source: based on Regional Technical Advisory group for Eastern Africa experts' opinion

5.2. Interpretation of results

The interpretation of results may include a discussion of trends, emerging resistance, difficulties and biases encountered, relevance of the findings and a comparison of the situation along the food chain. The following are covered in the WOAH 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 the epidemiological cut-off point) is preferred. This is based on the distribution of MICs or inhibition zone diametres 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 transmission from local, subnational and national levels – links to policy and interventions

Data should be in electronic format and stored in a structured data management system (e.g. Excel file, Laboratory Information Management System (LIMS), or any available data management software, such as WHONET) for ease of sharing and further data analysis. The data should be collected and analysed at local, subnational and national levels and presented in a consistent format grouped by target bacteria as follows:

All *Salmonella* spp.

i. *S. Enteritidis*
ii. *S. Typhimurium*
iii. Serovars other than *Salmonella Enteritidis* or *Typhimurium*

Escherichia coli

All *Campylobacter* spp.

i. *C. coli*
ii. *C. jejuni*

Staphylococcus aureus (MRSA)

All *Enterococcus* spp.

i. *Ent. faecalis*
ii. *Ent. faecium*

The following are also recommended in the region:

(i.) interpretive criteria used for minimum inhibitory concentration (MIC) determination if deviating from the recommended susceptibility testing methods;
(ii.) description of quality assurance systems;
(iii.) results of AST (MIC value);
(iv.) results of AST will be in table form for every animal population, broken down by bacterial species;

(v.) qualitative tables to report the result for each antimicrobial tested including
a. number of isolates tested,
b. number of resistant isolates,
c. number of fully susceptible and number of isolates resistant to 1, 2, 3, 4, 5 or >5 antimicrobials of different classes;
(vi.) prevalence measures for each organism and antimicrobial combination, preferably as a time series if there is enough data, including confidence intervals;
(vii.) quantitative tables to report MIC distributions for each animal species in each bacterial species;
(viii.) MIC_{50} and MIC_{90} calculations for each antibiotic;
(ix.) prevalence of resistant isolates in the target population and reported for the appropriate epidemiological unit, i.e. animals, flocks, food samples; and
(x.) confidence intervals of the prevalence values expressing the precision of the estimates.

See Tables 5.3, 5.4, 5.5 and 5.6 for examples.
Each country is encouraged to formally establish an AMR technical working group on surveillance that brings together microbiologists, epidemiologists and pharmacologists to ensure national sectoral and multisectoral analysis of the AMR data is collected in a manner that can easily be translated into policy and other interventions by the national AMR coordination committees.

Table 5.3 Example table for recording minimum inhibitory concentration 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	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)																		
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)																		
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**
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 for recording resistance (percentage) 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 (percentage) 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 for recording 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)
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 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 the 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 and/or 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 also be 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 Table to record multidrug-resistant *Salmonella* by animal source

Isolate source	**Poultry**	**Swine**	**Cattle**
Number of isolates tested	(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. Management of AMR data and epidemiological analyses of various antibiogram patterns using the WHONET software and the upcoming InFARM system and IT platform

The World Health Organization (WHO) Collaborating Centre for Surveillance of Antimicrobial Resistance at the Brigham and Women's Hospital in Boston, Massachusetts has developed an easy to use and freely downloadable software called WHONET that allows for antimicrobial resistance (AMR) data management and analysis in the human health sector. It includes various features, although not exhaustive, to accommodate the use of the software in the food and agriculture domains. WHONET offers modules for laboratory configuration, data entry, data analysis, and data encryption, among others. The software incorporates the latest CLSI and EUCAST human and animal breakpoints, as well as epidemiological cut-off values. The principal goals of this software are 2-fold: (i) to enhance the 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 national and international surveillance network. Currently, the software is available in 44 languages and is used in over 130 countries around the world managing data from over 2, 000 clinical, public health, veterinary and food laboratories.

WHONET analytical tools facilitate an 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. Currently, WHONET can handle results from testing bacteria, fungi and parasites, and virology is currently a priority area for further development. The WHONET interface permits many types of analysis including isolateline-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.

Additionally, WHONET includes a data import software called BacLink, which facilitates the capture and standardization of data from existing desktop applications, laboratory instruments, and laboratory information systems. This helps eliminate the need for duplicate data entry. Countries are utilizing BacLink to share standardized AMR data files with the Global Antimicrobial Resistance and Use Surveillance System (GLASS) developed by WHO.
Existing AMR data files (e.g. Excel files) can also be exported into WHONET using BacLink. Please note that the template provided in Annex 6 aligns with the data fields and configuration for WHONET for ease of use and collation of AMR data from multiple laboratories.

Through the FAO Action Plan on Antimicrobial Resistance 2021-2025 the Organization committed to developing a global epidemiological information system to regularly collect and analyze reliable and comparable AMR data in the food and agriculture sectors. In line with this commitment, FAO is currently finalizing the development of the International FAO Antimicrobial Resistance Monitoring (InFARM) system and information technology (IT) platform. The InFARM IT platform will assist countries in collecting, analyzing, and utilizing their AMR data from animals and food for national purposes. The InFARM IT platform will offer a secure repository for official national AMR data with restricted access and the option to share data at varying levels of confidentiality. The dashboards in the InFARM IT platform are being designed to provide interactive visualizations of AMR data in the different user interfaces. InFARM will also support countries willing to make their AMR data publicly available for global surveillance. InFARM will serve as the mechanism for contributing data from the food and agriculture sectors to the Quadripartite Global Integrated System for Surveillance of AMR and AMU (GISSA). FAO plans to issue a global call for AMR data early in 2024. This initial call

will initiate a continuous cycle for generating global AMR estimates related to food and agriculture on an annual basis, facilitated through subsequent yearly open calls. All countries will be invited to nominate InFARM focal points and submit AMR data during a designated period lasting several months, starting from the date of the open call.

In parallel, FAO is currently working with WHONET developers on updated training materials of a new version of WHONET that will be fully adapted to manage AMR data from the food and agriculture sectors. At the same time, FAO is working on the interoperability of WHONET and BacLink software with the InFARM IT platform.

This work will be crucial in facilitating the transmission of standardized AMR data files that comply with InFARM requirements during the forthcoming global open call for data.
Through the deployment of the FAO Assessment Tool for Laboratories and AMR Surveillance Systems conducted in various regions and countries, FAO has consistently highlighted the need for strengthening the management of national AMR data in the food and agriculture sectors. Additionally, epidemiological analyses reveal several facets of movement of resistant isolates between animals and between humans and animals that facilitate an understanding of the genesis and spread of antimicrobial resistance. These can be done manually but efficient data management and real-time analyses should be done with softwares and data platforms.

These guidelines encourage member countries to use WHONET for data management and the upcoming InFARM IT platform for global data sharing and analysis of AMR data to enable comparisons from various countries. More details are available at whonet.org/index.html, and https://www.fao.org/antimicrobial-resistance/resources/database/infarm/es/

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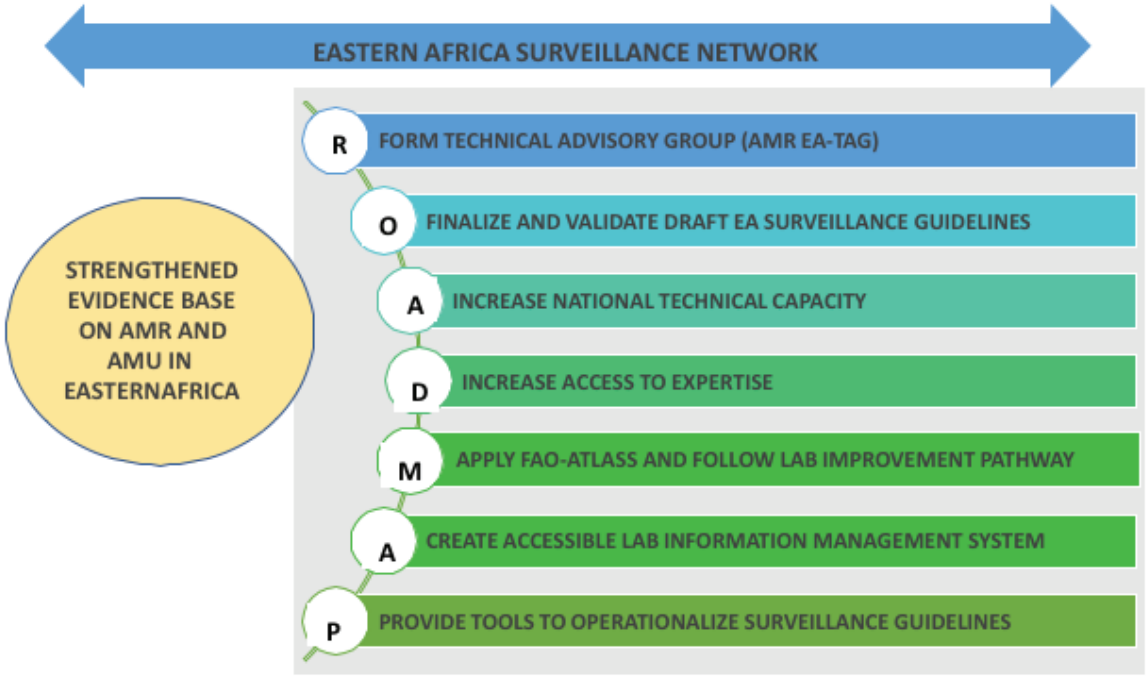
Chapter 6 - Regional roadmap for implementing these guidelines

6.1. The roadmap and key stakeholders

In 2019, FAO in collaboration with the World Organisation for Animal Health (WOAH), Africa Centers for Disease Control and Prevention, African Union – Interafrican Bureau for Animal Resources (AU- IBAR), International Livestock Research Institute (ILRI), East African Community (EAC), Intergovernmental Authority on Development Centre for Pastoral Areas and Livestock Development (IGAD-ICPALD), World Animal Protection (WAP), United States of America Centers for Disease Control and Prevention (CDC-Kenya), University of Nairobi and Kenya Medical Research Institute (KEMRI) facilitated countries to meet under the umbrella of an informal AMR network meeting which led to the delineation of a regional road map (Figure 6.1) to strengthen, coordinate and harmonize AMR surveillance in the region. The same agencies and countries together with the core members of the Technical Advisory Group for Eastern Africa (TAG-EA) (see Annex 1) will remain as the key stakeholders to support the implementation of these guidelines as well as the roadmap.

Figure 6.1 Regional roadmap to strengthen antimicrobial resistance surveillance in Eastern Africa



The aim of the roadmap is to set out the processes, tools, and coordination that should be undertaken at the regional level to support harmonization and implementation of the national AMR surveillance strategies and plans. These building blocks are interrelated and should be developed and implemented in parallel rather than sequentially by the stakeholders mentioned above. There should be partner capacity building (expert training and upgrade lab facilities) in subregional countries.

National governments, regional economic communities, international organizations, intergovernmental and non-governmental bodies, academia and donors can use this roadmap and guidelines to direct their capacity building activities within the region.

The first two milestones of the roadmap will be achieved upon validation and publication of these guidelines and therefore only the other milestones are described below.

6.1.1. Increase national technical capacity

A key challenge highlighted in the past is inadequate relevant expertise at the national level, including but not limited to clinical microbiologists and veterinary diagnosticians, veterinary epidemiologists with experience in developing AMR, AMU and AM residues surveillance programmes, technicians for maintaining vital laboratory equipment, pharmacists and chemists for implementing laboratory residues tests and medicine quality tests, communications specialists to support advocacy and awareness programmes, and IT support to develop and maintain laboratory data management. There is urgent need to improve the ongoing training and support available existing national technical staff to enable them to gain the relevant experience to implement activities to support national AMR and AMU surveillance processes, and to maintain their knowledge base in line with technical advances in future. Specific training needs that are a priority for regional harmonization include:

- Basic and advanced methods of antimicrobial sensitivity testing, including PCR and WGS, and on archiving of bacterial isolates;
- Biosafety and biosecurity in the laboratory, including management of laboratory waste; and
- Epidemiology, including field techniques (sampling schemes and sample handling for AMR tests), data analysis and reporting.

6.1.2. Develop regional procurement process

Countries have emphasized the significant challenges faced in ensuring a reliable supply of suitable quality bacteriology laboratory equipment and consumables. Shortages of consumables occur frequently within the Eastern Africa region which leads to delays in sample processing and potentially impacts on the quality of the data generated. Where equipment is present, a lack of replacement parts when mechanical or software failure is experienced can render the equipment unusable for long periods. In addition to low availability, the cost of purchasing consumables and equipment in the region is often disproportionate compared to other geographical regions.

Creating regional mechanisms to create a standardized supply pool of commercial AST materials could be possible through agreements with commercial partners to make available regionally standardized products (e.g. MIC plates for sensitivity testing against an agreed upon panel of antibiotics). Alternatively, procurement at regional level, supported for example by the EAC and facilitated through a regional reference laboratory (see below), could harness the power of bulk purchasing to reduce costs and to create a more secure and reliable source of supply for national laboratories.

6.1.3. Increase access to expertise

Better access to regional and international expertise could be achieved through:

(i) use of AMR reference centres;
(ii) creation of a regional laboratory network; and
(iii) access to external quality assurance.

AMR reference centres: FAO, WOAH, WHO have accredited several AMR references centres around the globe.

These centres will act as centres of technical excellence across terrestrial, aquatic animal health, plant health, food safety, apiculture, environment and waste management in line with the strategic objectives of AMR risk mitigation. The centres can play a role in providing expertise and training for national staff to strengthen laboratory, epidemiology and policy capacity including mentorship to support professional growth and help to minimize the brain drain from national facilities.
Regional laboratory network: Creating regional laboratory networks would improve access for national technical personnel to specialized equipment and expertise and would promote the sharing of data at the regional level. For example, designating regional reference centres for specific aspects of analysis, such as WGS, would increase access for countries within the region lacking such national facilities, and would also provide accessible and cost-effective knowledge exchanges and training options for national technical staff. Building stronger cooperation between laboratories within regions will also facilitate data sharing on AMR and collaborative risk assessment and management for new and emerging resistance within the region.

Access to external quality assurance: For surveillance data to be meaningful it must be produced via a quality assured process. National laboratories require access to an affordable external quality assurance (EQA) scheme through which they can obtain ongoing feedback to establish whether the national systems in place are sufficient. Options for EQA schemes within the Eastern Africa region are currently assumed to be limited, but should be explored through a review, which could be undertaken by or in conjunction with the TAG-EA.

The future aim is to have adequate capacity within the region, via regional reference laboratories, and preferably within each national network via national reference laboratories, for all laboratories that form part of a national surveillance system to participate in an EQA scheme that is compliant with internationally recognized standards. In the meantime, it is envisaged that the FAO AMR reference centres could provide this EQA facility to key national laboratories.

6.1.4. Apply FAO-ATLASS to identify gaps and monitor improvement

FAO has developed the Assessment Tool for Laboratories and AMR Surveillance Systems (FAO-ATLASS) 3 to assist countries in systematically assessing their AMR surveillance systems in food and agriculture. This tool has already been applied in Ethiopia, Kenya, the Sudan and the United Republic of Tanzania, and the findings have informed and contributed to the development of the roadmap.

FAO-ATLASS includes two modules – one for surveillance and one for laboratory assessment – that collect descriptive and semi-quantitative data to assess the performance of national AMR surveillance systems across five main pillars: (i) governance, (ii) data collection and analysis, (iii) data production network, (iv) communication, and (v) overall sustainability. Based on the assessment findings, a progressive improvement pathway stage is assigned to each AMR laboratory assessed in the country, to each pillar of the AMR surveillance and to the overarching AMR surveillance system of the assessed country. FAO-ATLASS allows national authorities to identify a strategic progressive approach to improving AMR surveillance systems and provides an evidence base for prioritizing capacity development action and for targeting advocacy. Implementation of FAO-ATLASS across a regional level can contribute to harmonization and better coordination of integrated AMR surveillance systems.

FAO has trained a pool of FAO-ATLASS assessors within the Eastern Africa region and continues to source external donor funding to implement national assessment missions on request from governments. Governments are also encouraged to self-fund assessments. Training national assessors permits countries to reapply the tool at regular intervals to measure their progress in addressing identified capacity gaps.

6.1.5. Develop low-cost accessible lab data management system

A Laboratory Information Management System (LIMS) allows veterinary laboratories to track samples from submission to testing and reporting. Through a shift from paperwork to computerized systems, LIMS can:

- facilitate the linkage between diagnostic results and timely response in the field, allowing informed prescribing based on sensitivity testing;
- facilitate provision of harmonized data to the national epidemiology unit in charge of data collection, collation and analysis for surveillance purposes; and
- provide information for action to risk managers and policy makers.

The more widespread the use of LIMS in national veterinary laboratories, the more homogenously information on animal diseases and on occurrence of resistance can be monitored and shared. More widespread implementation of LIMS in laboratories across the region will benefit the overall information management and flow of individual laboratories as well as the quality of the information collected.

Standardization of laboratory diagnostic processes and sample tracking is increasing in several African national veterinary laboratories through the use of a LIMS entitled SILAB for Africa (SILAB FA), established by the Experimental Institute for the Prevention of Animal Diseases of the Abruzzi and Molise “G. Caporale” and supported by FAO. Since 2012, SILAB FA has been installed in the national veterinary laboratories of a number of countries including but not limited to Botswana, Cameroon, Ethiopia, Kenya, Namibia, the United Republic of Tanzania, Zambia and Zimbabwe. The system is adapted to build on and coordinate with existing national systems.

Customization of the SILAB FA for AMR data has been initiated by FAO. The new module will be available to all countries that have already installed this LIMS. SILAB-FA is installed upon country request and is funded nationally. A low cost lite version of SILAB FA is under development to increase accessibility for resource constrained countries.

6.1.6. Create tools to support national strategy operationalization

There is a tendency for national AMR action plans and/or surveillance strategies, where they exist, to remain on paper with no progression to operationalization. Effective implementation of a national integrated One Health AMR surveillance system requires efforts at the national, regional and international levels. At the national level this requires a detailed assessment of the relevant stakeholders and existing systems, defined stakeholder roles and responsibilities, and an articulate plan for partnership, management, coordination and funding of surveillance structures. Currently, in many countries AMR data is generated mainly in the private sector, notably by large integrated intensive poultry and pig producers, and by academia.

The capacity, experience and expertise present in these sectors could be invaluable in supporting improvements in developing national surveillance systems, and the national operational plan (and advocacy strategy) should also reflect these stakeholders. The operational plan should encompass a review of the existing AMR and non-AMR surveillance programmes in each sector to assess whether AMR, AMU and residues surveillance can be embedded within these as a resource effective approach. It should review the national legislation framework to define the mandate for collection, sharing and analysis and action of AMR, AMU and residues data, and it should also be specific on funding requirements and on funding allocation (both from within government and external). Provision of a clear risk-based framework for prioritization of species, sector and locations (among other factors) for surveillance will support countries to focus limited resources more effectively.

At the regional and global levels, this process of operationalization can be facilitated by developing tools that provide a structured approach for countries to apply, considering lessons already learned by countries within the region. Already, some tools exist, such as:

- FAO/WOAH joint methodology to conduct analysis on the national legal framework relevant for AMU and AMR in the food and agriculture sector; and
- FAO's progressive management pathway on AMR (FAO-PMP-AMR). The tool helps countries tackle AMR step by step and in a way that allows them to find out where they stand in terms of managing AMR in food and agriculture sectors, identify the highest priority actions to be undertaken and track their progress.

6.1.7. Develop effective advocacy toolkit

To enable the operationalization process, policymakers must be convinced of the importance, not just of tackling the issue of AMR, but also specifically of the need for generating the national harmonized AMR, AMU and residues evidence base. A strong advocacy programme is required to engage key stakeholders within national governments.

6.2. Create an effective regional coordination mechanism: antimicrobial surveillance network

The Eastern Africa region has a regional platform to coordinate animal health issues – the Regional Animal Health Network that is comprised of the chief veterinary officers network (the umbrella) as well as regional epidemiology, laboratory and quarantine networks. The focal persons are drawn from national veterinary authorities. The network secretariat is hosted by the IGAD-ICPALD with the technical support of FAO. Recommendations to create an AMR subnetwork under RAHN have been floated before. Creating one specifically for AMR surveillance will ensure the coordination of approaches that have been suggested in these guidelines. It will also ensure that the date can be shared and analysed at the regional level to support regional actions by the IGAD-ICPALD and country partners. ICPALD and FAO will support the establishment and running of the network including development of terms of reference and a workplan.

6.3. Monitoring the implementation of the guidelines

The IGAD- ICPALD, FAO and the network will coordinate to ensure these guidelines are implemented in a phased approach and monitored including capacity building activities captured in this chapter. A monitoring and evaluation framework will be developed, and progress reported every year to the RAHN.

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Annex 2 - Livestock population in Eastern Africa

			Year				
Country	Species	**Units**	**2015**	**2016**	**2017**	**2018**	**2019**
Burundi	Cattle	Head	796 530	769 991	1 077 539	1 110 936	756 036
	Chickens	1000 Head	2 339	2 841	2 300	2 850	2 728
	Goats	Head	2 357 387	3 000 000	3 619 962	3,249,827	3 227 903
	Pigs	Head	529 998	561 184	618 176	774 689	804 013
	Sheep	Head	399 640	602 110	527 464	548 608	728 050
Djibouti	Camels	Heads	70 991	70 894	70 827	70 861	70 894
	Cattle	Head	302 416	300 421	300 061	300 195	300 328
	Chickens	1000 Head					
	Goats	Head	515 619	514 444	514 525	514 733	514 941
	Pigs	Head					
	Sheep	Head	469 095	469 163	468 782	469 055	469 329
Eritrea	Camels	Heads	370 469	374 261	379 559	383 855	388 152
	Cattle	Head	2 099 522	2 122 939	2 112 808	2 117 877	2 122 945
	Chickens	1000 Head	1 246	1 152	1 161	1 144	1 127
	Goats	Head	1 840 306	1 815 476	1 812 215	1 814 018	1 815 820
	Pigs	Head					
	Sheep	Head	2 308 548	2 418 779	2 415 896	2 435 935	2 455 974
Ethiopia	Camels	Heads	1 228 023	1 209 321	1 212 659	1 265 297	1 281 468
	Cattle	Head	57 829 953	59 486 667	61 037 330	62 706 486	63 284 177
	Chickens	1000 Head	60 506	59 495	59 887	62 106	64 455
	Goats	Head	29 704 958	30 200 226	30 789 034	33 061 808	34 045 216
	Pigs	Head	34 691	35 107	35 436	35 986	36 535
	Sheep	Head	28 892 380	30 697 942	31 840 682	31 562 151	31 849 003
	Horses	Head	2 082 203	2 158 176	2 226 036	2 282 996	2 319 656
	Mules	Head	405 950	409 877	372 978	304 588	307 820
Kenya	Camels	Heads	3 059 840	3 222 593	3 338 757	3 273 445	4 721 900
	Cattle	Head	18 728 076	20 529 190	18 338 810	19 635 142	20 898 769
	Chickens	1000 Head	41 472	43 796	48 125	52 219	56 659
	Goats	Head	25 094 383	26 745 916	25 684 489	26 710 775	35 172 749
	Pigs	Head	462 033	504 395	554 301	567 843	596 414
	Sheep	Head	16 795 198	18 983 760	18 759 072	19 485 699	27 440 945
	Horses	Head	2 053	2 060	2 089	2 112	2 137

Rwanda	Cattle	Head	1 349 792	1 214 244	1 166 187	1 293 768	1 330 192
	Chickens	1000 Head	4 837	5 238	5 273	5 442	5 566
	Goats	Head	2 706 382	2 605 780	2 923 706	2 731 795	2 685 038
	Pigs	Head	1 492 506	1 684 709	1 716 438	1 330 461	1 228 465
	Sheep	Head	716 309	637 068	664 703	601 836	587 949
Somalia	Camels	Heads	7 214 325	7 216 095	7 213 027	7 230 058	7 243 792
	Cattle	Head	4 889 370	4 850 000	4 800 000	4 746 819	4 232 413
	Chickens	1000 Head	3 675	3 692	3 667	3 694	3 721
	Goats	Head	11 646 431	11 501 714	11 473 213	11 432 240	11 466 768
	Pigs	Head	3 727	3 869	3 911	3 885	3 859
	Sheep	Head	12 039 813	11 583 732	11 000 000	10 457 119	11 687 422
	Horses	Head	855	889	887	893	899
	Mules	Head	22 015	22 167	22 107	22 156	22 206
South Sudan	Camels	Heads					
	Cattle	Head	11 823 700	11 830 800	11 837 500	12 074 116	13 143 378
	Chickens	1000 Head	15 000	15 000	15 000	15 000	15 000
	Goats	Head	13 551,667	13 568 846	13 564 517	13 572 317	13 580 117
	Pigs	Head					
	Sheep	Head	17 237 236	17 573 217	17 790 163	18 202 231	18 614 300
Sudan	Camels	Heads	4 809 000	4 830 000	4 850 000	4 872 000	4 895 000
	Cattle	Head	30 376 000	30 632 000	30 926 000	31 223 000	31 489 000
	Chickens	1000 Head	47 194	47 884	48 584	49 294	50 015
	Goats	Head	31 227 000	31 481 000	31 659 000	31 837 000	32 032 000
	Pigs	Head					
	Sheep	Head	40 210 000	40 612 000	40 752 000	40 846 000	40 896 000
Uganda	Cattle	Head	14 156 618	14 786 008	15 376 481	15 855 409	16 334 337
	Chickens	1000 Head	33 246	35 784	35 949	36 494	37 038
	Goats	Head	14 096 960	15 466 102	15 859 649	16 280 410	16 701 172
	Pigs	Head	2 461 661	2 631 033	2 648 977	2 697 715	2 746 453
	Sheep	Head	1 895 380	2 032 326	2 024 955	2 047 269	2 069 583
United Republic of Tanzania	Cattle	Head	26 713 644	26 935 923	26 519 776	27 282 702	27 821 063
	Chickens	1000 Head	36 554	37 272	37 518	37 992	38 472
	Goats	Head	18 026 051	18 779 631	18 017 462	18 497 912	18 918 816
	Pigs	Head	508 961	515 901	518 023	520 853	523 563
	Sheep	Head	6 168 295	5 751 090	7 332 299	7 945 775	8 291 176

Annex 3 - Proportion of live animals slaughtered annually

Country	Species					
	Camels	Chicken	Cattle	Goats	Pigs	Sheep
Burundi		298.7	7.4	19.0	18.2	14.9
Djibouti	6.4		18.1	35.8		8.9
Eritrea	4.1	135.1	10.9	39.4		27.8
Ethiopia	12.2	150.1	5.9	33.4	115.3	32.3
Kenya	7.2	54.9	13.4	20.8	65.2	15.5
Rwanda		114.8	29.1	22.9	21.5	17.6
Somalia	3.8	129.0	10.7	25.8	55.0	29.7
South Sudan		134.1	11.6	52.2		63.8
Sudan	11.0	71.9	11.2	41.8		37.9
Uganda		140.3	7.7	20.3	80.1	34.7
United Republic of Tanzania		273.0	11.8	19.0	71.7	27.3

Annex 4 - 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, which was referred to 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. The 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 for the calculation of the proportion of resistance to a particular antimicrobial drug in the relevant livestock sectors in the country, and for 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 prevalence should be covered, 50 percent prevalence is recommended 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 antimicrobial susceptibility testing (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, see 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 that 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

Expected prevalence [a]	90% Level of confidence Desired precision			95% Level of confidence 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	9.152
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

Source: WOAH. 2017. WOAH Terrestrial Manual 2017. Chapter 2.9.3. Paris, WOAH.
Note: a 50 percent is used when this is not known, or when there is intention to test multiple antibiotics which will otherwise have an array of expected prevalence for each type.

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 targeted bacteria. 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. Sometimes, 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 considered. 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 the 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

Note: 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. These include: Section 2.7.1 Health status of the animal sources; Section2.7.2 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 best to pool the samples for further microbiological analysis.

Annex 5 - 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 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 bacterial 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 should be calculated, taking into account the expected prevalence of the zoonotic pathogen. Examples for this adjustment are shown in Table A2.1 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]

Notes: [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 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 ten 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 must be increased according to the within-herd prevalence.

Example for antimicrobial resistance monitoring in Salmonella in farm animals year one

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

The required number of specimens = 384 x 2.5 = 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, i.e. 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 the presence of the pathogen on a randomly selected carcass needs to be taken into account. The lower this value is, 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 depending on the extent of 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 one. 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:

$$N = \frac{((Z_{alpha} \times \sqrt{2}\ pq) - (Z_{beta} \times \sqrt{p1\ q1 + p2q2}))2}{(p1 - p2)2}$$

Formula 2 (Dohoo, Martin and Stryne, 2009) is applicable:
Where N = Total bacterial isolates to be subject to AST per year, $p=(p_1+p_2)/2$, p_1 = prevalence year 1, p_2 = prevalence year 2, $q=1-p$, $q_1 = 1-p_1$, $q_2=1-q_2$, Z_{alpha}= typically set at 95 percent confidence level ($z=1.96$), Z_{beta} =typically set at 80 percent confidence level ($z=-0.84$).

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

Annex 6 – Regional template for the surveillance of antimicrobial resistance in agriculture

Identification number	Country	admin level 1	admin level 2	admin level 3	admin level 4	Village	Farm number	Location type[1]	Location	Species[2]	Breed	Animal type[3]	Age[4]	Age category[5]	Market category[6]	Food

Notes:
[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

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

Part 3: Data inputs for antibiotic 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 coding 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, 2, 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 export
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 not there yet	

Annex 7 - List of Standard Operating Procedures to Support these Guidelines

The following list of standard operating procedures (SOPs), in addition to what is already incorporated into the guidelines document, have been attached as part of the guidelines for antimicrobial resistance (AMR) surveillance and monitoring. Countries may adapt these and use according to the facilities and technologies available for processes from sampling to culture, antimicrobial susceptibility testing (AST) and data analysis and dissemination.

1. SOP: Sample collection by different sample types
2. SOP: Sample transportation
3. SOP: Sample reception and transfer at laboratory
4. SOP: Bacteria isolation
5. SOP: Sample processing (culture) and detection of bacteria (biochemical testing of food-borne pathogens)
6. SOP: Serological tests for bacteria identity confirmation
7. SOP: Antimicrobial susceptibility testing and reporting by disk diffusion
8. SOP: Antimicrobial susceptibility testing and reporting by minimum inhibitory concentration (MICs)
9. SOP: Antimicrobial susceptibility testing by polymerase chain reaction (PCR) detection of extended-spectrum β-lactamases (ESBLs)
10. SOP: Laboratory biosafety and biosecurity
11. SOP: Boot swab removal
12. SOP Quality assurance and control for culture, identification and antimicrobial susceptibility testing
13. SOP: Validation of selective MacConkey agar plates supplemented with 1 mg/L cefotaxime for monitoring of ESBL- and AmpC producing *E. coli* in meat and caecal sample
14. SOP: *S. aureus* agglutination test
15. SOP: Methodology and specific primer sets for each species
16. SOP: Elimination method for *S. aureus*
17. SOP: Phenotypic testing for *E. coli*
18. SOP: Laboratory biosafety manual
19. SOP: Isolation of methicillin-resistant *Staphylococcus aureus* (MRSA) from food-producing animals and farm environment
20. SOP: Validation of selective and indicative agar plates for monitoring of carbapenemase-producing *E. coli*
21. SOP: Isolation of ESBL-, AmpC- and carbapenemase-producing *E. coli* from caecal samples
22. SOP: Isolation of ESBL-, AmpC- and carbapenemase-producing *E. coli* from fresh meat
23. SOP: Quantification of ESBL/AmpC-producing *E. coli* in caecal content and fresh meat samples

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