

INTEGRATED SURVEILLANCE OF ANTIMICROBIAL RESISTANCE IN FOODBORNE BACTERIA

Application of a One Health Approach



World Health
Organization

Integrated Surveillance of Antimicrobial Resistance in Foodborne Bacteria: Application of a One Health Approach

**Guidance from the WHO Advisory Group on Integrated
Surveillance of Antimicrobial Resistance (AGISAR)**

In collaboration with
Food and Agriculture Organization of the United Nations (FAO), and
World Organisation for Animal Health (OIE)



**World Health
Organization**

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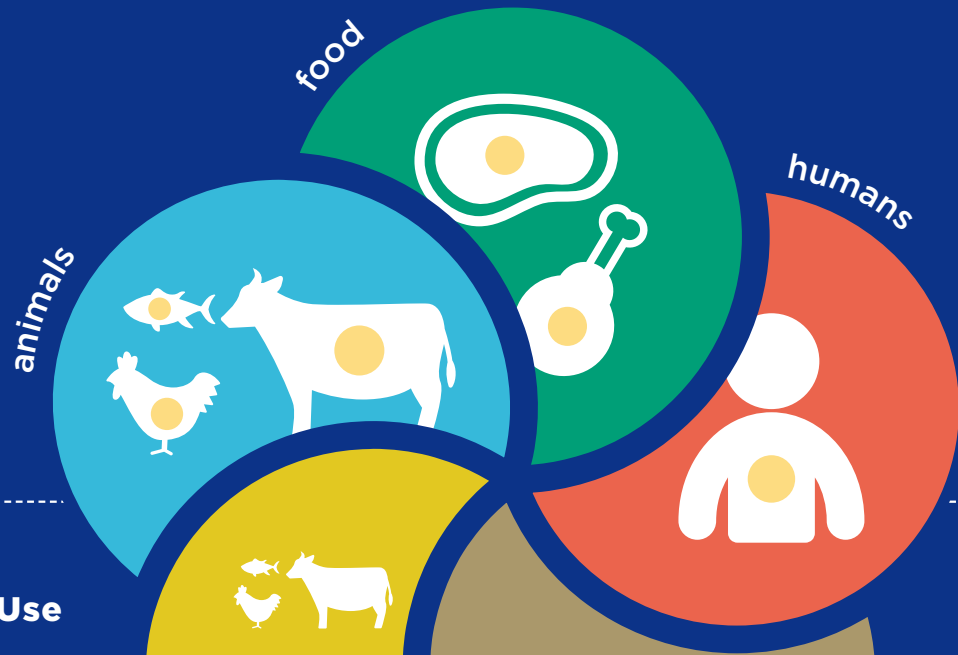
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Integrated Surveillance of Antimicrobial Resistance in Foodborne Bacteria

Surveillance of Antimicrobial Resistance



Surveillance of Antimicrobial Use








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
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Declaration of Interest

All experts and resource advisors invited to participate in the Expert Consultation completed the WHO standard form for declaration of interest prior to the meeting. At the start of the meetings, all participants were asked to confirm their interest, and to provide any additional information relevant to the subject matter of the meeting. No conflicts of interest were identified.

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Acronyms and abbreviations used in this document

AGISAR	Advisory Group on Integrated Surveillance of Antimicrobial Resistance
AMR	Antimicrobial resistance
AST	Antibacterial susceptibility testing
ATC	Anatomical Therapeutic Chemical
ATC-vet	Anatomical Therapeutic Chemical classification system for veterinary medicinal products
CDC	US Center for Disease Control and Prevention
CIPARS	Canadian Integrated Program for Antimicrobial Resistance Surveillance
CLSI	Clinical and Laboratory Standards Institute
DDDA	Defined daily dose animals
DCDvet	Defined course dose veterinary medicines
DDDvet	Defined daily dose for veterinary medicines
ECDC	European Centre of Disease Prevention and Control
ECOFF	Epidemiological resistance cut-off value
EFSA	European Food Safety Authority
EMA	European Medicines Agency
EQAS	External quality assurance programme
ESAC-Net	European Surveillance of Antimicrobial Consumption Network
ESBL	Extended spectrum beta-lactamase
ESVAC	European Surveillance of Veterinary Antimicrobial Consumption
EU	European Union
EUCAST	European Committee on Antimicrobial Susceptibility Testing
FAO	Food and Agriculture Organization of the United Nations
GFN	WHO Global Foodborne Infections Network
GLASS	Global Antimicrobial Resistance Surveillance System
GLOBAL-PPS	Global Point Prevalence Survey of Antimicrobial Consumption and Resistance
HAI-Net	Healthcare Associated Infection Network
ISO	International Organization for Standardization
JIACRA	Joint Interagency Antimicrobial Consumption and Resistance Analysis
MALDI	Matrix-assisted laser desorption/ionization
MARAN	Dutch Monitoring of Antimicrobial Resistance and Antibiotic Usage in Animals
MIC	Minimum inhibitory concentration
MLST	Multilocus sequence typing
NARMS	National Antimicrobial Resistance Monitoring System for Enteric Bacteria
NethMap	Netherlands Human Antimicrobial Resistance Surveillance
NORM-VET	Norwegian Antimicrobial Resistance Surveillance Programme
OIE	World Organisation for Animal Health
PCR	Polymerase chain reaction
PCU	Population correction unit
PFGE	Pulsed-field gel electrophoresis
PMSs	Practice Management Systems
WGS	Whole genome sequencing
WHO	World Health Organization

Introduction

The purpose of this guidance is to assist WHO Member States, and other stakeholders, in the establishment and development of programmes of integrated surveillance of antimicrobial resistance in foodborne bacteria (i.e., bacteria commonly transmitted by food). In this guidance, “integrated surveillance of antimicrobial resistance in foodborne bacteria” is defined as the collection, validation, analyses and reporting of relevant microbiological and epidemiological data on antimicrobial resistance in foodborne bacteria from humans, animals, and food, and on relevant antimicrobial use in humans and animals. Integrated surveillance of antimicrobial resistance in foodborne bacteria therefore includes data from relevant food chain sectors (animals, food and humans) and includes data on both antimicrobial resistance and antimicrobial use. Integrated surveillance of antimicrobial resistance for foodborne bacteria expands on traditional public health surveillance to include multiple elements of the food chain, and to include antimicrobial use data, to better understand the sources of infection and transmission routes.

Antimicrobial resistance increases mortality, morbidity and health expenditures in humans and animals. Inappropriate use of antimicrobials in humans and animals contributes to increasing antimicrobial resistance. In 2015, the Sixty-eighth World Health Assembly adopted the global action plan on antimicrobial resistance¹ aimed at combating the increasing health threat posed by antimicrobial resistance. Recognizing the urgent need for multisectoral action to address antimicrobial resistance, the governing bodies of the Food and Agriculture Organization of the United Nations (FAO) and the World Organisation for Animal Health (OIE) also adopted resolutions supporting the global action plan on antimicrobial resistance in 2015.

Tackling the public health threat posed by antimicrobial resistance requires effective antimicrobial resistance surveillance programmes. The essential need for robust antimicrobial resistance surveillance systems is emphasized in the Global Action Plan on Antimicrobial Resistance. However, according to the *Worldwide Country Situation Analysis: Response to Antimicrobial Resistance*² published by WHO in 2015, few countries worldwide have adequate surveillance systems for antimicrobial resistance and antimicrobial use in health care settings, in the community, in the environment, and across the food chain. Furthermore, few countries have mutually comparable surveillance approaches, adequate coordination, or sufficient data sharing between human and animal sectors. Countries often have ineffective public health antimicrobial resistance surveillance systems because they lack antimicrobial resistance expertise, have poor laboratory infrastructure, and have inadequate data management capacity.

Illustrating its commitment to multisectoral efforts to combat antimicrobial resistance, WHO established the WHO Advisory Group on Integrated Surveillance of Antimicrobial Resistance (AGISAR) in 2008. AGISAR supports WHO’s and Member States’ efforts to minimize the public health impact of antimicrobial resistance associated with the use of antimicrobials in food-producing animals. AGISAR brings together, in addition to the antimicrobial resistance focal points from FAO and OIE, internationally renowned experts in a broad range of disciplines relevant to antimicrobial resistance appointed through a transparent selection process.

1 www.who.int/antimicrobial-resistance/publications/global-action-plan/en/

2 www.who.int/antimicrobial-resistance/publications/situationanalysis/en/

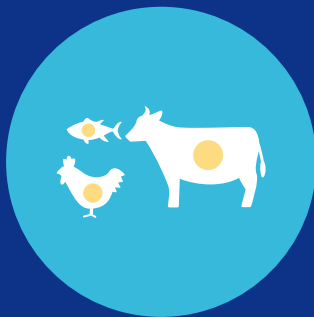
The need for this guidance was identified in the first AGISAR strategic plan adopted at the first AGISAR meeting in 2009. In 2013, after a consultative process of four years, WHO published the first version of *Integrated Surveillance of Antimicrobial Resistance: Guidance from a WHO Advisory Group*³. At the sixth AGISAR meeting in 2015, a second AGISAR strategic plan was established. The central theme of this second AGISAR strategic plan was to identify how AGISAR will support WHO and Member States in the implementation of the WHO global action plan on antimicrobial resistance. The revision of this guidance was identified as among the key actions to be accomplished in the upcoming year.

Similar to the 2013 guidance, the present revised guidance provides the basic information that countries need in order to establish programmes of integrated surveillance of antimicrobial resistance in foodborne bacteria. This guidance describes a step-by-step approach to designing a programme of integrated surveillance of antimicrobial resistance in foodborne bacteria and includes recommended standardized and validated antimicrobial susceptibility testing methods, harmonized interpretive criteria, and approaches to the collection and reporting of antimicrobial consumption and use data. Chapter 1 provides guidance on surveillance approaches for tracking antimicrobial resistance in foodborne bacteria and includes minimum requirements for a programme of integrated surveillance of antimicrobial resistance in foodborne bacteria. Chapter 2 offers guidance on surveillance approaches for tracking antimicrobial use. Chapter 3 touches upon combined analysis and reporting of a programme of integrated surveillance of antimicrobial resistance in foodborne bacteria.

3 http://apps.who.int/iris/bitstream/10665/91778/1/9789241506311_eng.pdf

1

Surveillance of Antimicrobial Resistance





1. Surveillance of antimicrobial resistance

1.1. Scope

A programme of integrated surveillance of antimicrobial resistance in foodborne bacteria includes the coordinated sampling and testing of antimicrobial susceptibility of bacteria from food-producing animals, food, and humans using epidemiological (including sampling) and microbiological methods that enable comparison of results. Use of comparable epidemiological and microbiological methods is necessary to allow comparison of antimicrobial susceptibility results between different areas, countries and regions. An important impediment to comparing antimicrobial susceptibility results is the lack of uniform standards and policies in sampling, testing, and reporting across countries and sectors. Using comparable epidemiological, microbiological, and reporting methods does not mean that all surveillance systems must conduct their activities in exactly the same way. Local epidemiology, public health resources, laboratory capacity, government policies, production practices, food processing, distribution of food products, and pre-existing public health infrastructure may influence the design of an antimicrobial resistance surveillance programme. The objectives and targets that are defined in the National Action Plan to address antimicrobial resistance inform the objectives and implementation steps of the programme of integrated surveillance of antimicrobial resistance in foodborne bacteria. Since programmes of integrated surveillance of antimicrobial resistance in foodborne bacteria are likely to vary between countries, a clear description of the empirical design, sampling, and testing methods should be provided so that the strengths and limitations of each programme can be assessed.

This chapter aims to describe appropriate design elements, sampling approaches, microbiological methods, and reporting procedures for a programme of integrated surveillance of antimicrobial resistance in foodborne bacteria so that surveillance is conducted, and results are reported, in a comparable fashion. Concretely, this chapter:

- provides guidance on the minimum requirements and design of a programme of integrated surveillance of antimicrobial resistance in foodborne bacteria;
- provides guidance on sampling strategies;
- provides guidance and standards for laboratory methods including primary isolation, bacterial identification, antimicrobial susceptibility testing, and quality assurance;
- proposes analysis and reporting methods that allow findings to be compared within and between countries; and
- makes recommendations for international harmonization of programmes of integrated surveillance of antimicrobial resistance in foodborne bacteria, including both pathogenic and commensal bacteria.

While antimicrobial susceptibility testing of bacteria isolated from humans has been conducted since antimicrobials first became widely available, it was initially limited to local programmes designed to guide patient therapy. As resistance to new antimicrobials emerged, and multiple drug resistance emerged and spread, the need for comprehensive antimicrobial resistance surveillance programmes that provide timely information to enable public health interventions and prevent emergence and spread of antimicrobial resistance was recognized as a public health priority throughout the world.

To be most effective, surveillance of antimicrobial resistance in foodborne bacteria requires an integrated approach using comparable methods. In 2000, a WHO report recommended that

countries develop programmes of integrated surveillance of antimicrobial resistance in foodborne bacteria that include antimicrobial susceptibility data from bacterial isolates originating from patients, food-producing animals and, where appropriate, retail meats (1). The World Organisation for Animal Health (OIE) has developed standards on antimicrobial resistance surveillance in animals, which are published in the Manual of Diagnostic Tests and Vaccines for Terrestrial Animals (2), Terrestrial Animal Health Code (3) and the Aquatic Animal Health Code (4). A key attribute of a programme of integrated surveillance of antimicrobial resistance in foodborne bacteria is the capacity to monitor and detect the emergence and spread of resistant bacteria in animal products and other foods destined for human consumption. The increasing global trade in food-producing animals and their derived commodities highlights the growing importance of global data-sharing on foodborne pathogens and disease, including data on antimicrobial resistance.

A programme of integrated surveillance of antimicrobial resistance in foodborne bacteria should provide data that can be used to:

- provide accurate estimates of the prevalence of antimicrobial resistance in different reservoirs;
- identify antimicrobial resistance trends over time and from place to place;
- describe the spread of resistant bacterial strains and genetic determinants of resistance;
- detect rare phenotypic or genetic traits (i.e. novel resistant strains or resistance genes);
- study the association between antimicrobial resistance and use of antimicrobial agents;
- generate hypotheses about sources and reservoirs of resistant bacteria;
- identify and evaluate the effectiveness of interventions to contain the emergence and spread of resistant bacteria;
- inform risk analysis of foodborne antimicrobial resistance hazards;
- guide evidence-based policies and guidelines to control antimicrobial use in hospitals, communities, agriculture, aquaculture, and veterinary medicine; and
- support educational efforts aimed at mitigating current and emerging hazards.

The design of comparable programmes of integrated surveillance of antimicrobial resistance in foodborne bacteria presents several challenges. Countries vary widely in their public health infrastructure, agricultural production systems and practices, food supply systems, and veterinary services. Therefore, to achieve comparability between programmes of integrated surveillance of antimicrobial resistance in foodborne bacteria, it is necessary to establish a minimum set of criteria for every programme. Conditions that will help facilitate the establishment of an effective programme of integrated surveillance of antimicrobial resistance in foodborne bacteria include: 1) an adequate health care and veterinary services infrastructure that allows human and animal clinical specimens to be properly collected and microbiological culture to be performed as part of routine care; 2) established human health, veterinary, or food safety laboratory facilities and trained laboratory personnel; 3) laboratory quality management systems; and 4) capacity to capture, validate, analyse and report surveillance data.

A programme of integrated surveillance of antimicrobial resistance in foodborne bacteria must provide data for public health decision making. The sustainability of a surveillance programme is directly associated with the ability of this programme to provide accurate data needed for public health decision making in a timely manner. The participation of different sectors and disciplines is particularly helpful in sustaining the programme of integrated surveillance of antimicrobial resistance in foodborne bacteria. Scientists and professionals from different disciplines (e.g. physicians, veterinarians, microbiologists, epidemiologists and environmental scientists), and representatives from food production industries, as well as government agencies responsible for risk assessment, risk management and research, have a role in supporting and sustaining a programme of integrated surveillance of antimicrobial resistance among foodborne



bacteria. In addition to a sound surveillance infrastructure, including an appropriate sampling design and adequate microbiological, epidemiological, and data management capacities, a sustainable programme of integrated surveillance of antimicrobial resistance in foodborne bacteria is commonly accompanied by: 1) continued political and financial support arising from a recognition of the public health importance of antimicrobial resistance and antimicrobial use surveillance; 2) ongoing quantitative and qualitative risk assessments for emerging and potential hazards and the flexibility to adjust resources and priorities as necessary; 3) cooperation and good communication between the public health, animal health and agriculture sectors, and between microbiologists, clinicians, epidemiologists, veterinarians, food scientists, environmental scientists, food producers and public health officials; 4) timely and effective publication of findings for different audiences; and 5) a continuous process for programme review and enhancement. Furthermore, a well-functioning programme of integrated surveillance of antimicrobial resistance in foodborne bacteria provides an excellent foundation for a robust microbiological and epidemiological research agenda to better interpret the antimicrobial resistance data.

1.2. Elements of a programme of integrated surveillance of antimicrobial resistance in foodborne bacteria

A programme of integrated surveillance of antimicrobial resistance in foodborne bacteria comprises the following elements:

A. Sample sources:

- Human specimens
- Retail food samples
- Samples from food-producing animals

B. Target bacteria

- Most commonly included bacteria
- Other bacteria

C. Sampling design

- Sample sources
- Sample information
- Sampling strategy

D. Laboratory testing methodology

- Bacterial culture and isolate identification
- Characterization of isolates
- Standardized antimicrobial susceptibility testing
- Quality control
- Recommended antimicrobials for surveillance
- Characterization of isolates

E. Data management, validation, analysis and reporting

- Minimal data elements
 - Human isolates
 - Food-producing animals isolates
 - Retail food isolates
- Interpretation of antimicrobial susceptibility results

- Software tools
- Data analysis and reporting

1.3. Sample sources

Testing of bacteria isolated from at least the following three sources is optimal for a programme of integrated surveillance of antimicrobial resistance in foodborne bacteria: 1) human (people in healthcare facilities and in the community), 2) retail food (animal-derived food products), and 3) food-producing animals (sick and healthy). Isolates from all sources should be tested for antimicrobial susceptibility using recognized and comparable methods, and using similar antimicrobial agents. Depending on available resources, a programme of integrated surveillance of antimicrobial resistance in foodborne bacteria can be implemented incrementally or using focused priority study populations.

1.3.1 Human specimens

Monitoring bacterial isolates from ill persons should be the first priority in a programme of integrated surveillance of antimicrobial resistance in foodborne bacteria. Isolates from such specimens may be acquired from health facilities with laboratory capacity for routine clinical testing. Isolates obtained for antimicrobial resistance surveillance should include representative isolates from sporadic and outbreak foodborne disease cases. It may be helpful to distinguish isolates collected from persons several days after hospitalization, which may represent healthcare associated infections, from isolates collected from ill persons in the community or upon admission to a healthcare facility. Most foodborne illnesses in humans result in diarrhoea, therefore, stool specimens are the most common sources of bacterial pathogens in some countries. Because of the large number of cases of foodborne illnesses, testing a subset of the isolates from persons with foodborne illnesses may be sufficient for antimicrobial resistance surveillance. However, because extra-intestinal infections due to foodborne bacterial pathogens are less common and are associated with higher morbidity and mortality, it may be desirable to test a higher proportion of isolates from extra-intestinal infections. Routine surveillance may be expanded with surveys and research projects involving selected subpopulations (e.g. older people, young people and healthy carriers). Guidance on conducting antimicrobial resistance surveillance among isolates from humans is provided by the WHO Global Antimicrobial Resistance Surveillance System (GLASS)¹.

1.3.2 Retail food samples

Retail food is a second priority specimen to include in a programme of integrated surveillance of antimicrobial resistance in foodborne bacteria. Food of animal origin represents the major route of human exposure to foodborne pathogens with antimicrobial resistance. The selection of foods for surveillance (beef, chicken, turkey, pork, etc.) should reflect consumption patterns in the population and likely prevalence of antimicrobial resistance, but may be modified from year to year in order to capture multiple commodities. It is helpful to collect food samples that reflect the purchasing habits of the consumer (e.g. in open markets or chain stores). The statistical database of the FAO (5) summarizes consumption data for different countries and is a useful source of information to help determine food sampling priorities.

¹ www.who.int/antimicrobial-resistance/publications/surveillance-system-manual/en



1.3.3 Samples from food-producing animals

Collecting samples from food-producing animals should be a third priority for a programme of integrated surveillance of antimicrobial resistance in foodborne bacteria. Although samples from both healthy animals and sick animals are useful for surveillance, samples from healthy animals should be the primary focus for surveillance because such samples can provide an unbiased measure of antimicrobial resistance in source animals for the human food supply. Samples collected from food-producing animals should be taken from the same animal species as the retail meat food samples collected for the programme of integrated surveillance of antimicrobial resistance in foodborne bacteria.

1.4. Target bacteria

The selection of the type of bacteria (genus and species) to be included in a programme of integrated surveillance of antimicrobial resistance in foodborne bacteria depends on the public health priorities, antimicrobial use practices, and the estimates of the burden of foodborne illnesses (6).

1.4.1 Most commonly included bacteria

Worldwide, *Salmonella* is usually the first priority for inclusion in a programme of integrated surveillance of antimicrobial resistance in foodborne bacteria. *Campylobacter* spp. is also an important foodborne pathogen and is commonly included in programme of integrated surveillance of antimicrobial resistance in foodborne bacteria. Programmes of integrated surveillance of antimicrobial resistance of foodborne bacteria may also include other bacteria. Because *Escherichia coli* are common and some strain variants may cause disease, *E. coli* can be used as a sentinel organism for antimicrobial resistance. *E. coli* and *Enterococcus* spp. also serve as reservoirs of resistance genes that can be transferred to human pathogens transiting the intestinal tract; as such, they provide information on the flow of Gram-negative and Gram-positive resistance traits in the food chain.

1.4.2 Other bacteria

The choice of other bacteria depends on the epidemiology of foodborne diseases in the area, which may change over time. In addition to the major pathogens mentioned above, other bacteria (e.g. *Staphylococcus* and *Clostridium*) may be relevant, including those associated with aquaculture (e.g. *Vibrio*).

1.5. Sampling design

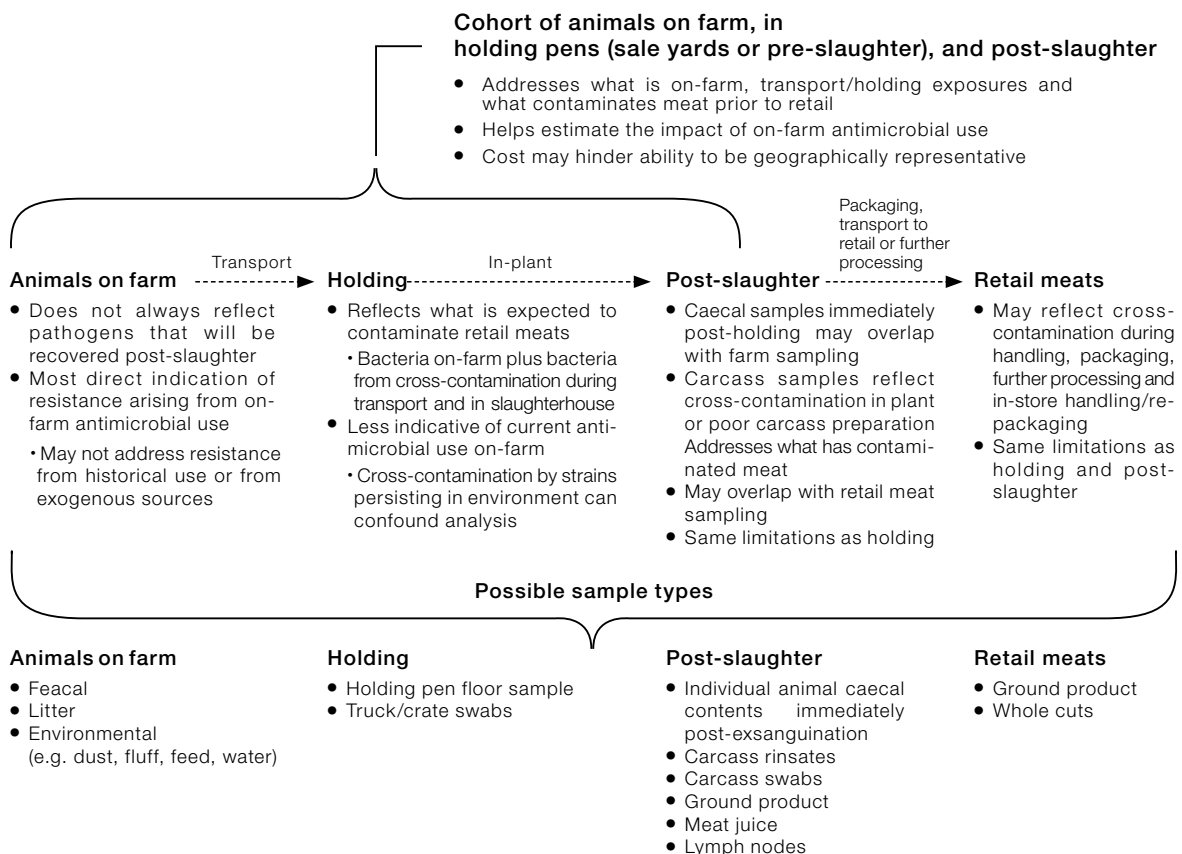
The methods whereby samples are collected along the food chain, particularly sampling from food-producing animals, can impact the reliability of inferences made from that component of the programme of integrated surveillance of antimicrobial resistance in foodborne bacteria.

1.5.1 Sample sources

When developing the sampling design for selecting which bacterial isolates derived from specimens collected from ill persons to include in a programme of integrated surveillance of antimicrobial resistance in foodborne bacteria, usually either all the available isolates or a

random sample of the isolates (with each isolate having an equal chance of selection) are included. Sampling from healthy people and retail foods requires more attention to sampling design particularly when attempting to interpret the public health significance of detected antimicrobial resistance. For food-producing animals, the impact of sampling design on interpretation of antimicrobial resistance results is more complex because there are many potential sampling points in the production and processing continuum, and different information will be obtained at different points (7). When reporting surveillance data, sufficient information on the sampling strategy should be provided to allow interpretation of results and comparisons with other programmes of integrated surveillance of antimicrobial resistance in foodborne bacteria that may have different sample collection points. Fig.1.1 provides an overview of sampling considerations at different points along the food animal production continuum. In general, sampling at the production site (e.g. on farms or in aquaculture facilities) will yield bacteria most directly associated with the antimicrobial use environment, but these may not reflect the bacteria surviving processing and reaching the food supply. Environmental sampling (e.g. composite chicken litter samples) may be considered as an alternative to individual animal sampling when necessary, as long as representativeness has been established. Bacterial isolates, including *Salmonella* serotypes, in an animal vary with time and place in the production chain (8,9). Other factors that may affect results include season, latitude, processing methodology, transportation and storage. Slaughterhouses are usually the most convenient and affordable point for collection of animal samples. It is generally preferable to collect caecal samples, although this option may be limited by practical difficulties or cost. Caecal samples generally provide a higher recovery of isolates than carcass sampling, and better reflect farm-level exposure in individual animals (by reducing the likelihood of contamination from the processing environment). It should be noted that the microbiota of the animal caecum may be affected by the time spent in transport and in holding pens, and the persisting microorganisms that can be acquired in each environment (10).

Fig.1.1 Examples of sampling considerations through the production to postharvest continuum





1.5.2 Sample information

It is important to record basic information on each sample. This will allow more comprehensive analysis of laboratory data, help clarify potential biases for different sample types, and help identify critical control points for mitigating antimicrobial resistance emergence and spread. For isolates from humans, the following basic information should be included with each specimen: age (or date of birth), sex, date of specimen collection, specimen type, geographical location (town or city, state or province, etc.), hospitalization status, and, if hospitalized, date of admission to the hospital. Other useful information that could be obtained from sentinel sites or during special studies may include recent travel history, previous and current antimicrobial use, immune status, whether the sample was collected as part of an outbreak investigation and, if so, any data from the investigation, including the known or suspected food source. For isolates from retail foods, the following information should be included with each specimen: date, type of store and location, type of food (raw, or processed, or ready-to-eat), animal species, processing plant identification, origin (imported or domestic), whether fresh or frozen, organic, conventional or other production system, and if the food was prepackaged or subject to in-store processing. Most information can be captured simply by filing a copy of the package label. For food animal samples collected during production, each sample should include the following information: animal species, date and place of collection, state or country of origin (imported or domestic), age and clinical status of the animal, and possibly the history of antimicrobial use in animals and on the farm. Additional information on food animal samples should include whether the sample was from ill or healthy animals, and from an individual animal or a pooled sample from several animals. For samples collected at slaughter, information may include the state or country of origin of the animal (domestic or imported), slaughter class (e.g. dairy or beef cattle), the processing plant, age of animal, source of the specimen (rectal swab, caecal sample, etc.) and, if possible, the address or postal code of the farm of origin.

1.5.3 Sampling strategy

The relative strengths and limitations of sampling methods should be considered when establishing a programme of integrated surveillance of antimicrobial resistance in foodborne bacteria and when interpreting and comparing results. Sampling may be active (prospective) or passive (samples collected for other purposes), random or systematic, statistically-based or convenience-based. Sentinel surveillance, which relies on specific providers, healthcare facilities, laboratories, or other sources reporting a disease or condition under surveillance, may also be employed. If sentinel laboratories are used for provision of data or isolates for the integrated antimicrobial resistance surveillance system, data from sentinel laboratories may include antimicrobial susceptibility results. Sentinel surveillance requires fewer resources and is often more complete and timely than population-based surveillance, but it may not be representative of the entire population. In order to permit analysis of antimicrobial resistance trends, sampling should be done on a continuous or regular basis using consistent methods.

For surveys and periodic surveillance studies, the frequency of testing should be decided on the basis of the incidence and seasonality of the bacteria or diseases under surveillance. In some established programmes of integrated surveillance of antimicrobial resistance in foodborne bacteria, samples are collected monthly. If resources are not adequate for such frequent testing, isolates should be collected periodically throughout the year from different sites, in sufficient numbers, to identify trends. Several statistical methods can be used to calculate the number of isolates needed for testing (sample size). Sample size will depend on the desired precision for estimates of the prevalence of resistance and the magnitude of change in resistance to be detected over a specified period of time in a certain population (denominator). Sample size also depends on the initial or expected prevalence of resistance and the size of the population to be monitored. Furthermore, sample size also depends on the desired level of statistical

significance and power to detect a difference. The European Food Safety Authority has compiled tables showing required sample sizes for different antimicrobial resistance monitoring programme objectives (11).

1.6. Laboratory testing methodology

Laboratories providing isolates or antimicrobial susceptibility results for a programme of integrated surveillance of antimicrobial resistance in foodborne bacteria should be able to isolate target bacteria from different specimen types, and identify bacteria to the genus and species levels using internationally accepted microbiological methods. It will also be helpful for participating laboratories to be able to determine pathotypes of *E. coli* using validated methods and perform antimicrobial susceptibility testing using validated methods according to established standards such as those of the Clinical and Laboratories Standards Institute (CLSI) or the European Committee on Antimicrobial Susceptibility Testing (EUCAST). It is also helpful for participating laboratories to be able to (or have access to a reference laboratory that is able to) determine serotypes of *Salmonella* and characterize isolates using molecular methods such as PCR and sequencing, PFGE, multilocus sequence typing (MLST) and whole genome sequencing (WGS). WHO capacity building activities, such as the WHO Global Foodborne Infections Network (GFN) or AGISAR projects, help provide technical support and training in food microbiology to participants. In addition, participation in an external quality assurance programme (EQAS), such as WHO GFN's EQAS (12,13) or others, is recommended.

1.6.1 Bacterial culture and isolate identification

Different recovery methods can be used based on the type of samples (e.g. food samples) which can differentially enrich bacterial subpopulations within a sample. However, it is important to be aware that media selecting for resistance (e.g. broth with cefotaxime to select for ESBL-producing bacteria) leads to different recovery rates than non-selective media. Culture methods and media should meet recognized international laboratory standards. As with other design considerations, culture methods should be defined beforehand and be described in surveillance reports. Differences in culture methodology should be taken into account when data from different surveillance programmes are compared. Monitoring laboratories are encouraged to collaborate with established monitoring systems, national reference laboratories, WHO Collaborating Centres and other partners to provide long-term storage for a representative number of isolates that can be used for future testing and analysis. Bacteria should be identified to the species level using standard methods (conventional or automated biochemical tests, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry [MALDI], or any other validated methods). For some species, it will be necessary to identify the bacteria to the type level (e.g. serotype, MLST, etc.).

1.6.2 Standardized antimicrobial susceptibility testing

Only in vitro antimicrobial susceptibility testing methods that have been standardized and validated under the auspices of an internationally recognized consensus standards organization, such as CLSI or EUCAST, should be used. This is a critical feature of a sound antimicrobial resistance surveillance programme, and is the only way to ensure reliable data. The steps in these official standards should be strictly followed and should not be modified for local use. Standard breakpoints for other bacteria of interest not described in this guidance can be found in CLSI documents; M100S, M45, or VET01-S2 or via the web site of the EUCAST². See Appendix 1 for a description of the differences between the two systems.



EUCAST and CLSI standards cover test performance and interpretation for both disk diffusion and minimum inhibitory concentration (MIC) methodologies. In either case, quantitative results (disk diffusion zone diameters or MIC values) should be measured and recorded, in addition to the categorization of an isolate as susceptible or resistant. Tracking changes in the distribution of quantitative results can be very helpful in following bacterial resistance patterns over time, and also allows retrospective data analysis if breakpoints or cut-off values are changed. Susceptibility testing methods for *Salmonella* and *E. coli* are well known and widely available. Validated testing methods for *Campylobacter* were developed more recently and are less widely known. CLSI has established the quality of a disk diffusion method for screening isolates for resistance to erythromycin (15 µg disk) ciprofloxacin (5 µg), and tetracycline (30 µg) where growth up to the disk (i.e. no zone of inhibition) indicates acquired resistance determinants in *Campylobacter* that correlate with tentative resistance breakpoints (14). A disk-based method has been used by EUCAST to establish disk diffusion clinical and epidemiological cut-off values (ECOFFs) using the same erythromycin, ciprofloxacin and tetracycline disk load. However, EUCAST ECOFFs were established using a different test medium and incubation conditions (15), but the same quality control organism (*C. jejuni* ATCC33560). No other method of disk diffusion testing has been formally validated for *Campylobacter*, although comparison studies have been conducted. For *Campylobacter* testing by broth microdilution, a CLSI testing method is available for amoxicillin, ampicillin, azithromycin, chloramphenicol, ciprofloxacin, clindamycin, doxycycline, erythromycin, florfenicol, gentamicin, nalidixic acid, streptomycin, telithromycin and tetracycline.

1.6.3 Quality Control

Quality control (QC) testing and frequency should follow international guidelines. Expert rules for discordant susceptibility results, as published by CLSI, should be applied to ensure data integrity. Infrequent resistance to a specific antimicrobial should always be re-tested to ensure validity of the result. The presence of contaminants, incorrect identification of bacteria, misreading of growth, lack of knowledge about how to read results, user error and the use of non-validated methods are the most common reasons for failed quality control testing results (see Appendix 2 for more information).

1.6.4 Recommended antimicrobials for surveillance

The selection of antimicrobials to be included in a programme of integrated surveillance of antimicrobial resistance in foodborne bacteria will depend on the target organism and the purpose. Some antimicrobial agents are clinically relevant, while others are included due to epidemiological importance. For *Salmonella*, streptomycin resistance is useful for tracking certain strain types, for example, *Salmonella* serotype Typhimurium DT104; however, susceptibility results are often unreliable due to overlapping susceptible and resistant populations. Trimethoprim and sulfamethoxazole are tested separately by some programmes for epidemiological purposes whereas they are tested combined for clinical purposes. *Enterococcus* is often used to monitor resistance to antimicrobial agents with Gram-positive activity. The selection of antimicrobials has been based on a variety of well-established surveillance and monitoring programmes. This selection of antimicrobials provides a harmonized standard which enables comparison across laboratories and countries. The antimicrobials listed in Table 1.1 could be considered for testing *Salmonella*, *E. coli*, *Campylobacter* spp., *Enterococcus* spp., and *Staphylococcus* spp. Interpretive criteria according to CLSI and EUCAST are listed in Appendix 3. See also the pathogen-antimicrobial combinations selected by GLASS for global reporting of antimicrobial resistance in humans and WHO priority list of

pathogens³.

1.6.5 Characterization of isolates

Characterization of foodborne bacterial isolates (genus, species, and additional microbial subtyping) is important but it depends on the capacity of the laboratory. If participating laboratories do not have capacity for characterization of the isolates, they can send their isolates to a reference laboratory for characterization. Serotype information is fundamental to understanding the epidemiology of *Salmonella* and its multidrug resistant isolates. However, not all laboratories would necessarily test for all possible serotypes of *Salmonella*. The most common serotypes in a given area should be known in order to ensure an adequate supply of antisera (16). For epidemiological purposes, it may also be helpful to type the isolates using molecular fingerprinting techniques such as PFGE, MLST or WGS. For antimicrobial resistant pathogens having public health implications, it would be helpful to have isolates characterized for resistance mechanisms using PCR/sequencing, WGS, etc.

1.7. Data management, validation, analysis and reporting

Reporting results from a programme of integrated surveillance of antimicrobial resistance in foodborne bacteria should include comprehensive analyses of surveillance data from all sources. Rigorous, ongoing validation of laboratory and sample data is essential. On a routine basis, a joint evaluation of the data by surveillance system experts is recommended. Furthermore, feedback on surveillance results should be solicited from microbiologists, epidemiologists, veterinarians, clinical practitioners and food scientists representing all sectors of the programme of integrated surveillance of antimicrobial resistance in foodborne bacteria. Depending on the size of the programme of integrated surveillance of antimicrobial resistance in foodborne bacteria, it can be advantageous to appoint a national coordinating body to audit and evaluate the integrated surveillance findings. The coordinating body should organize and direct the analysis to help ensure that the integrated analysis, reporting and risk communication are done properly and in a timely manner. This group can also ensure that the programme continues to meet the intended public health needs as outlined in the programme scope. They can also recommend modifications to address emerging issues. It is important that the data are analysed with an emphasis on the human health significance of the findings. Surveillance results should be transparent and easily accessible. The results should also be communicated in language that can be understood by non-specialists. It is helpful to compose narrative summaries, written in plain language, to accompany the data, in order to help consumers and other stakeholders understand the risks, hazards, and meaning of significant or notable trends. The core of any programme of integrated surveillance of antimicrobial resistance in foodborne bacteria is an isolate-level database containing relevant details of demographic (epidemiological data) and microbiological characteristics of samples. Data should be stored in a secure centralized database that permits simple data entry and retrieval, as well as flexible reporting of standard and ad hoc analysis results. Compatibility with similar databases at the national and international level facilitates collaboration among networks and systematic comparison of findings.

³ For pathogen-antimicrobial combinations selected by GLASS for global reporting of antimicrobial resistance in humans, see: www.who.int/antimicrobial-resistance/publications/surveillance-system-manual/en/



Table 1.1 Suggested antimicrobials, by bacteria, for inclusion for antimicrobial susceptibility testing in a programme of integrated surveillance of antimicrobial resistance in foodborne bacteria

Antimicrobial classes	<i>Salmonella, E. coli</i>	<i>Campylobacter</i> ⁱ	<i>Enterococcus</i>	<i>Staphylococcus</i>
Aminoglycosides	Gentamicin	Gentamicin Streptomycin	Gentamicin Streptomycin	Gentamicin
Amphenicols	Chloramphenicol		Chloramphenicol	Chloramphenicol
Carbapenems	Imipenem Meropenem			
Cephalosporins II	Cefoxitin			Cefoxitin
Cephalosporins III	Cefatoxime (or Ceftriaxone) Ceftazidime			
Cephalosporins IV	Cefepime ⁱⁱ			
Glycopeptides			Vancomycin <i>Teicoplanin</i>	Vancomycin
Glycylcyclines	<i>Tigecycline</i>		Tigecycline	
Lincosamides		<i>Clindamycin</i> ⁱⁱⁱ		Clindamycin
Lipopeptides			Daptomycin	
Macrolides	Azithromycin	Erythromycin ^{iv}	Erythromycin	Erythromycin
Nitrofurans	<i>Nitrofurantoin</i> ^v		<i>Nitrofurantoin</i> ^{vi}	
Oxazolidinones				Linezolid
Penicillins	Ampicillin Amoxicillin <i>Temocillin</i> ^{vii}	Ampicillin	Ampicillin	Penicillin Oxacillin
Polymyxins	Colistin			
Quinolones	Ciprofloxacin Nalidixic acid <i>Pefloxacin</i> ^{viii}	Ciprofloxacin <i>Nalidixic acid</i> ^{ix}	Ciprofloxacin	Ciprofloxacin
Rifamycins				Rifampicin
Streptogramins			Quinupristin-dalfopristin	Quinupristin-dalfopristin
Sulfonamides ^x	Sulfisoxazole ^x			Sulfisoxazole
Tetracyclines	Tetracycline	Tetracycline ^{xi}	Tetracycline	Tetracycline
Trimethoprim ^x	Trimethoprim			Trimethoprim

Antimicrobials italicized are second priority.

- i Recommended antimicrobials used for surveillance of *Campylobacter jejuni* and *Campylobacter coli*.
- ii Cefepime is used in the European Union (EU) to distinguish between AmpC and ESBL.
- iii Lincosamides are used in the treatment of some infections caused by *Campylobacter*.
- iv Resistance towards erythromycin reflects azithromycin resistance.
- v Nitrofurantoin is used in the United States of America for testing Gram-positive bacteria.
- vi It is optional for *Enterococcus* to be tested for nitrofurantoin.
- vii Temocillin is included as a marker to detect the *bla_{OXA-48}* genotype.
- viii To screen for ciprofloxacin resistance in *Salmonella* spp. when disk diffusion is used.
- ix Nalidixic acid is used in *Campylobacter* to identify rare mutations.
- x Trimethoprim-sulfamethoxazole can be used instead of using sulfisoxazole or trimethoprim alone.
- xi Doxycycline may be used instead of tetracycline.

1.7.1 Minimal data elements

The elements to be collected in a programme of integrated surveillance of antimicrobial resistance in foodborne bacteria should reflect the specific scientific and public health objectives, and should take into account the feasibility of consistent collection of the desired fields. Consequently, it is not possible to define a single universal list of minimal data elements. However, this section presents items that can serve as a basis for consideration by programme directors and data managers. In many instances, data are entered manually into the surveillance data management system. In other instances, a laboratory may already have a data management system or laboratory instrument system for recording test results. In that case, electronic transfer of results from the routine data management system to the surveillance system is highly recommended, in order to avoid time-consuming and error-prone manual re-entry of existing electronic data. Irrespective of the source of a sample, the following data elements would be useful for inclusion in the surveillance protocol and database design: 1) sample information: common sample identifier, date of sample collection, type of sample, basic epidemiological data, geographic site of collection; 2) culture results: microbial species identification, and, where relevant, serotype; 3) antimicrobial susceptibility test results: antimicrobials tested, susceptibility test methods, quantitative susceptibility test results, and qualitative test interpretations such as resistant, intermediate, and susceptible; and 4) additional relevant laboratory tests performed.

While it is possible to conduct a programme of integrated surveillance of antimicrobial resistance in foodborne bacteria without quantitative test results, the scientific and epidemiological value of the resulting data will be significantly compromised. Quantitative results give insights into the population ecology and mechanisms of resistance, as well as data quality that are not possible with test interpretation categories of resistant, intermediate, and susceptible. Furthermore, these interpretive categories are generally determined using clinical interpretation breakpoints rather than epidemiological cut-off values (ECOFFs), which can mask significant changes in the molecular epidemiology of resistance. Clinical breakpoints may also change over time as knowledge of treatment outcomes improve and dosages change. Long-term surveillance should not be linked to breakpoints at a given point in time, but instead, data should be re-interpreted retrospectively as dictated by the most current interpretive criteria.

1.7.1.1 Human isolates

In studies of isolates of bacteria from humans, the study population usually includes ill individuals presenting to healthcare facilities for diagnosis and therapy. Alternatively, some special studies may focus on bacterial colonization or carriage, either in healthy individuals or in patients. Possible data fields to be considered, including all the data previously mentioned under “sample information” are: 1) patient identifiers including medical record number, national identification number, and patient name; 2) patient demographics such as date of birth or age, sex, race, ethnicity, or nationality; 3) patient location such as medical ward, clinic, the location where the specimen was collected from the patient, or patient’s place of residence; and 4) clinical information such as medical diagnosis and epidemiological risk factors (e.g. international travel or food exposures).

1.7.1.2 Food-producing animal isolates

Samples from food-producing animals can be collected at many points in the food production process. Samples can be taken from healthy animals on farms or at slaughter. Animal isolates from food-producing animals may be collected from samples already being gathered to satisfy regulatory requirements, surveillance protocols, or to diagnose sick animals. Possible data fields to be considered for inclusion are: 1) animal identification information including herd number, pen number, and animal identification number; 2) animal demographic information

including animal species and production class; and 3) animal location.

1.7.1.3 Retail food isolates

Food isolates may be collected from investigations of suspected foodborne outbreaks, to satisfy regulatory requirements, or to support defined surveillance protocols. In surveillance programmes exploring the links between antimicrobial resistance elements in food animals and humans, the focus is generally on food of animal origin. In other instances, it may also be of interest to collect samples from foods of plant origin. Possible data fields to be considered for inclusion are: 1) food sample identifiers; 2) food demographics (animal or plant species); 3) food location (location of food collection); and 4) sample and retail establishment characteristics (store type).

1.7.2 Interpretation of antimicrobial susceptibility results

Quantitative susceptibility test results, specifically disk diffusion zones of inhibition and minimum inhibitory concentration (MIC) values, have a potential to provide greater insight into the molecular epidemiology of resistance characteristics than simple categorical interpretations (resistant, intermediate, and susceptible). Quantitative measurements have a number of critical benefits:

- evaluation of data quality;
- flexible analysis and re-analysis of data using different interpretative guidelines (CLSI vs. EUCAST, clinical vs. epidemiological interpretative criteria, changes in interpretative guidelines over time);
- phenotypic characterization of isolates based on their level of resistance;
- discrimination between distinct microbial subpopulations; and
- evaluation of the adequacy and robustness of reference interpretation criteria

To ensure harmonized reporting of surveillance data and facilitate comparison of results, it is recommended that ECOFFs be used when interpreting the results of in vitro antimicrobial susceptibility tests (17). It is also important to consider the clinical breakpoints provided by CLSI or EUCAST in order to evaluate the public health risk associated with the microorganism of interest and mechanism of resistance. ECOFFs are the MICs that distinguish strains with an acquired decrease in susceptibility (non-wild-type populations) from wild-type susceptible populations. Classifying strains relative to wild-type populations provides a relatively stable and discrete reference point for tracking changes in susceptibility over time. This approach also permits direct comparison of data from different surveillance systems with different clinical breakpoints. Because ECOFFs are empirically determined from a representative distribution of MIC values in the target population, this approach also largely avoids the need to re-analyse historical data when clinical breakpoints change (as often occurs when new clinical data are collected). The use of ECOFFs is also beneficial when no breakpoints have been formally established from clinical outcome data. It is important to note that the use of ECOFFs has led to confusion over the definition of resistance. This has traditionally been defined clinically as a means of predicting the likelihood of success of antimicrobial therapy. Historically, the resistant category has been established using extensive data sets that combine pharmacological parameters and clinical outcome studies with MIC data from wild-type populations. For this reason, it has been recommended that the term resistant be reserved for cases where clinical breakpoints have been formally established following clinical trials (18). As a minimum, in reporting, the way in which the term is used should be clarified to avoid misunderstanding. Most commonly, categories are determined using clinical interpretative guidelines, as published by CLSI or EUCAST. However, interpretation of results in terms of ECOFFs can provide a more accurate estimate of the emergence of resistance elements in a study population than



therapy-based predictors of clinical efficacy in humans. CLSI recommends that, in the absence of changes to the susceptibility test methodology, test results, even for historical data, should be interpreted using recent breakpoints rather than those available at the time the test was originally performed (19). The rationale for this recommendation is that clinical breakpoints more accurately reflect current understanding of clinical test interpretation and dosing regimens.

Data analysis software should have a variety of analysis options to permit the flexible exploration of resistance characteristics and associations. The following are some examples of analyses to be considered:

- Isolate listing: the user should be able to generate a list of isolates with specific sample or microbiological characteristics (e.g. animal species, time of collection, serotype or fluoroquinolone-resistance). It would be valuable to have a list of microbiological alerts to automatically flag organisms with unlikely, infrequent, or important resistance phenotypes.
- Isolate listing summary: it is also often of interest to calculate isolate total statistics lists in a manner which permits organisms to be tracked by time of collection, geographical location, animal species, or other parameters of interest.
- Percent resistant, percent intermediate, and percent susceptible: the most common way to present the results of antimicrobial susceptibility testing is as percentages of resistant, intermediate, and susceptible isolates. Such results can be stratified by time of collection, geographical location, animal species, and other characteristics to highlight changes over time or differences in study populations.
- Distribution of susceptibility test measurements: evaluation of data quality, flexible analysis and re-analysis of data using different interpretation guidelines (CLSI vs. EUCAST, clinical vs. ECOFFs) or when interpretative guidelines change over time; phenotypic characterization of microbial subpopulations and resistance traits; and evaluation of the adequacy and robustness of reference interpretation criteria.
- Scatterplots: comparison of the resistance findings between two antimicrobials permits an exploration of co-resistance (correlated resistance results) and cross-resistance (a specific type of co-resistance, in which resistance can be attributed to a single genetic mechanism).
- Multidrug resistance: the comparison of test results for several antimicrobials provides improved phenotypic characterization of resistance mechanisms and refines the discrimination of phenotypic subpopulations.
- Automated cluster detection: the routine application of statistical algorithms to datasets can be useful in the timely detection of clusters of identical or closely related isolates. Additionally, automated cluster detection can be useful in outbreak investigations.

1.7.3 Software tools

WHONET is freely available software for the management of microbiology test results. This software was developed and supported in 1989 by the WHO Collaborating Centre for Surveillance of Antimicrobial Resistance at the Brigham and Women's Hospital in Boston, United States of America. The software is currently in use in hospital, public health, veterinary, and food laboratories in over 110 countries, and is available in over 20 languages. This software and educational tutorials can be downloaded from www.whonet.org. WHONET includes the following modules:

- Laboratory configuration: characteristics of the laboratory, antimicrobials tested, locations for monitoring human isolates (e.g. hospital wards, clinics, communities), locations for monitoring animal isolates (e.g. farms, abattoirs, zoos), locations for monitoring food isolates (e.g. markets, restaurants), and configurable lists of optional data fields to be used for data entry.
- Data entry: the user enters information on the human, animal, or food subject of study

including relevant demographic and location details, sample information, microbial species, antimicrobial susceptibility test results, and clinical or molecular details.

- Data analysis: options include isolate listings and summaries, percentage resistant, test measurement statistics, scatterplots, multidrug resistance profiles, and statistical and microbiological alerts to possible outbreaks and important or unusual laboratory findings. Results can be saved as Microsoft Excel or Access files, which is particularly convenient when WHONET is run in automated batch mode.
- BacLink: this is the data import module for WHONET, which allows data to be transferred electronically rather than entered manually. Sources of data may include computer applications (Microsoft Excel, Microsoft Access, text files), laboratory test instruments, or commercial or in-house developed laboratory information systems.

1.7.4 Data analysis and reporting

To provide context for the surveillance findings, the programme structure and methodology should be described in sufficient detail to permit others to make sound comparisons with other programmes and their results. This should include: a description of the sampling design and specimen collection; the microbiological methods used for culture, identification and susceptibility testing; the interpretative criteria used for reporting; quality control and quality assurance measures; a glossary of terms; statistical methods; and any changes made in the methodology over time. Centralized databases should be designed in a way that allows data to be extracted appropriately and uniformly. For ease of analysis and reporting, data should focus on individual isolate identifiers with links to metadata, including denominator data. The database needs to be centrally managed. Also, data should remain confidential when shared with analysts. Where possible, surveillance data should be analysed in conjunction with other available datasets, such as information on antimicrobial use, pulsed-field gel electrophoresis (PFGE), MLST, PCR/sequencing of resistance genes, WGS, plasmid typing data (or other strain typing data), as well as outbreak investigations involving isolates recovered in surveillance. Additional information on the design of AMR surveillance systems and analysis of data is provided by the Clinical and Laboratory Standards Institute (20). Once data integrity and confidentiality have been ensured, data should be made freely available for independent analysis and reporting in as close as possible to real time.

1.8. Establishing and improving programme of integrated surveillance of antimicrobial resistance in foodborne bacteria

In establishing a programme of integrated surveillance of antimicrobial resistance in foodborne bacteria, resources generally go initially to designing, coordinating and implementing the system, designing valid sampling and culture methods, establishing partnerships to acquire samples, securing reagents for culture and instruments to conduct routine testing, validating and analyzing the data, and developing expertise through training. Once these fundamental components are in place, other goals of integrated surveillance can be considered. These goals include the following:

- Increase the timeliness of data collection and reporting. Data collection should occur at least annually, although not necessarily for all target organisms and all study populations.
- Establish avenues of cooperation, communication and data publication between agencies and disciplines.
- Report the analyses of emerging and ongoing human public health issues related to resistant pathogens.
- Carry out research to support and develop surveillance, identify intervention points, and



track the spread of resistance genes between ecological niches.

- Collect and report subtyping data (e.g. PFGE, genomic sequence) for serotypes with important resistance patterns.
- Periodically evaluate the surveillance methods used and the data collected to ensure that they are the most useful for public health purposes; make adjustments to address emerging hazards such as other pathogens and commodities.
- Improve methods, but ensure that improvements do not compromise comparisons with historical data.
- Collaborate with colleagues in other countries to ensure that new methods are adopted in a way that enables and encourages comparison of data among countries.
- Report temporal and spatial data on resistance together with data on antimicrobial use in humans and animals, to help increase understanding of practices that may contribute to resistance.

Programmes of integrated surveillance of antimicrobial resistance in foodborne bacteria exist in several countries. These programmes can be used as models for new programmes of integrated surveillance of antimicrobial resistance in foodborne bacteria. Examples of programmes already in place around the world include:

- Danish Integrated Monitoring Programme (DANMAP)
- US National Antimicrobial Resistance Monitoring System (NARMS)
- Canadian Integrated Programme for Antimicrobial Resistance Surveillance (CIPARS)
- Dutch Monitoring of Antimicrobial Resistance and Antibiotic Usage in Animals (MARAN)
- Netherlands' Human Antimicrobial Resistance Surveillance (NethMap)
- Norway's NORM-VET Programme
- Swedish Veterinary Antimicrobial Resistance Monitoring (SVARM) Programme
- National Antimicrobial Resistance Monitoring Programme (NARMP) in the Republic of Korea

These and other programmes of integrated surveillance of antimicrobial resistance in foodborne bacteria listed in the report of the first meeting of AGISAR (21).

Whole genome sequencing (WGS) combined with bioinformatic tools are now being used to monitor antimicrobial resistance. In recent years, whole genome sequencing WGS has become increasingly more affordable. In some countries, using WGS costs less than using conventional microbiology, including isolation, detection and molecular typing. There are free bioinformatics tools available online which have been developed for detection and typing of all microorganisms⁴. Several online tools created for the detection of antimicrobial resistance genes have been used for genotypic monitoring of antimicrobial resistance. The results of these monitoring efforts have been in approximately 99% concordance with the phenotypic data. For programmes of integrated surveillance of antimicrobial resistance in foodborne bacteria, WGS will most likely replace conventional laboratory methodologies in the future (see Appendix 4).

1.9. References

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2

Surveillance of Antimicrobial Use



2. Surveillance of Antimicrobial Use

2.1. Background

This chapter aims to support and promote the collection and reporting, at national and local levels, of comparable data on antimicrobial use in animals, particularly food-producing animals, and humans. Several international organizations have emphasized the importance of surveillance of the use of antimicrobials. WHO Global Principles on use of antimicrobials in food-producing animals state that authorities “should establish systems to determine the amounts of antimicrobials given to food animals”. The WHO Global Principles also state that “information on the amounts of antimicrobials given to food animals should be made publicly available at regular intervals, be compared to data from surveillance programmes on antimicrobial resistance, and be structured to permit further epidemiological analysis” (1).

In 2001, a WHO consultation on surveillance of the use of antimicrobials provided guidance on monitoring the use of antimicrobials and concluded that surveillance of the use of antimicrobials is needed for: 1) policies for the containment of antimicrobial resistance; 2) comparison of the use of antimicrobials at local, regional, national, international levels; 3) education of stakeholders; 4) correlation with data from antimicrobial resistance monitoring in humans, animals, and food; 5) application of risk analysis processes pertaining to the issue of antimicrobial resistance; and 6) evaluation of the impact of implementation of the prudent use of antimicrobials and of other interventions (2). Furthermore, the global action plan on antimicrobial resistance adopted by the World Health Assembly in 2015 and endorsed by United Nations Food and Agriculture Organization (FAO) and the World Organisation of Animal Health (OIE), concluded that antimicrobial use is a main driver for antimicrobial resistance, and improved antimicrobial use in both humans and animals is needed to combat antimicrobial resistance. In 2016, WHO released a methodology for monitoring national consumption of antimicrobial agents in humans (3).

A surveillance system of the use of antimicrobials in animals and humans should be multi-faceted and multi-sectorial. The OIE also provides guidance for the monitoring of antimicrobial use in food-producing animals (4) and aquatic animals (5). In 2015, OIE mandated that all OIE Member Countries gather data on the use of antimicrobial agents in food animals, and has created a global database for monitoring the use of antimicrobial agents in animals (6).

Surveillance of antimicrobial use and consumption can be divided into three main activities: 1) measuring the quantity of antimicrobials sold; 2) collecting information on prescribing of antimicrobials; and 3) collecting information on the actual intake of antimicrobials by humans or animals. In this chapter, the quantities of antimicrobials sold are referred to as antimicrobial consumption whereas the quantities of antimicrobials prescribed or taken in by humans or animals are referred to as antimicrobial use. Antimicrobial consumption data refer to estimates derived from aggregated data sources such as import or wholesaler data, or aggregated health insurance data where there is no information available on the patients who are receiving the medicines or why antimicrobials are being used. Antimicrobial use data refers to actual or estimated quantities derived from patient-level data. Antimicrobial use data often includes information on the individual patients (human or animal) and the indication for treatment.

Monitoring antimicrobial consumption (quantity of antimicrobials sold) provides an overall summary of the quantities of antimicrobials used at the country level. This is useful to describe quantities and classes of antimicrobials consumed in various parts of the health care and animal



sectors in order to identify patterns of use and trends over time. Monitoring antimicrobial consumption, which can usually be accomplished with minimal expense, is often done at the country level. Monitoring antimicrobial consumption can also be applied to sub-national regions and even to healthcare facilities or farms. If antimicrobial consumption is monitored using comparable methods, including the denominator (population, biomass), it is then possible to compare antimicrobial consumption between different countries, regions and individual facilities. Such data on antimicrobial consumption may be collected at different parts of the drug supply chain such as at import, wholesalers, pharmaceutical companies, hospitals, pharmacies, veterinary practices, and farms. Monitoring antimicrobial consumption should be done on a regular basis ideally through an annual monitoring programme collecting overall sales of antimicrobials at a country level.

Monitoring antimicrobial use (quantity of antimicrobial prescribing or drugs taken) provides additional and complementary information on how antimicrobials are actually used.

Antimicrobial use data, for instance, includes information about the patient or animal and the reason for treatment (indication or diagnosis). Collection of antimicrobial use data, usually by specific surveys, is resource-intensive. Consequently, antimicrobial use data, in contrast to antimicrobial consumption data, are usually collected intermittently (e.g. not collected on a regular basis) and are usually collected at the local level (hospitals, pharmacies, veterinary practices, farms) and rarely at the scale of a country.

Stakeholders utilize data on antimicrobial consumption and antimicrobial use for different purposes (see Table 2.1). Data on antimicrobial consumption and antimicrobial use are important for documenting the current situation and in raising awareness of antimicrobial use and resistance among authorities and the general public. Furthermore, collection and analysis of this data provides the opportunity to evaluate the impact of management measures such as benchmarking. This allows for the comparison of statistics on antimicrobial consumption or use with best practices, or between countries, regions, hospitals, veterinary clinics, farms, etc.

2.2. Surveillance of the use of antimicrobials in humans

A variety of approaches are available for conducting surveillance of the use of antimicrobials in humans, which vary in purpose, setting, methodology and output. These approaches include surveillance of national antimicrobial sales data, point prevalence surveys on antimicrobial use in hospitals, and longitudinal studies on antimicrobial use in hospitals or in the community. Point prevalence surveys, particularly if reported over time, are a simple and inexpensive way of identifying prescribing trends, linking results to antimicrobial resistance data, and identifying areas for improvement. Longitudinal studies, although more labour intensive, allow prospective audits of consumption of antimicrobials with direct interaction and feedback to prescribers. This strategy has proven effective for improving antimicrobial prescription and reducing costs. Given the quantity of antimicrobials used in hospitals, and the impact of antimicrobial resistance in these settings, it is recommended that surveillance of the use of antimicrobials in hospitals be given priority.

When conducting surveillance of the use of antimicrobials in humans, it is important to ensure that information on individual patients is kept confidential. In many countries, privacy laws require individual patient consent or the approval of an ethics committee before this type of information is collected. In any case, all patient data must be anonymous. Participants also need to be assured that individual hospital names will not be revealed in any internal or external report.

Table 2.1 Activities that can be achieved at the national level depending on the type of antimicrobial data collected

Application of surveillance data	Awareness raising	Identify temporal or regional trends	Evaluation of management measures	Integrated analysis with AMR data	Bench-marking
Level/type of data	At country level				
Level/type of data					
Overall national antimicrobial consumption	Yes	Yes	Yes	Yes	Yes
National consumption per healthcare sector (community and hospital)	Yes	Yes	Yes	Yes	Yes
National consumption by gender/age	Yes	Yes	Yes	Yes	Yes
Antimicrobial consumption and use in all/representative sample of healthcare facilities	Yes	Yes	Yes	Yes	Yes
Antimicrobial consumption and use in a limited number of healthcare facilities	Yes	No*	No*	No*	No*
Animals					
Overall national antimicrobial consumption	Yes	Yes	Yes	Yes	Yes
National antimicrobial consumption by animal species	Yes	Yes	Yes	Yes	Yes
National antimicrobial consumption by repartition**	Yes	Yes	Yes	Yes	Yes
Antimicrobial consumption and use in all/representative sample of farms overall or within a production sector (e.g. poultry farms)	Yes	Yes	Yes	Yes	Yes
Antimicrobial consumption or use in a limited number of farms (or veterinary clinics)	Yes	No*	No*	No*	No*

* These activities are still relevant for the individual facilities (hospitals or farms) where surveys are carried out even if this will not be representative at national level.

** Stratification of sales based on estimates of use by animal species



Surveillance programmes monitoring the use of antimicrobials in humans should, at a minimum, include antimicrobial agents that are classified as J01 (antimicrobials for systemic use) according to the Anatomical Therapeutic Chemical (ATC) classification system (7). Other groups of antimicrobials, such as antimicrobial agents for intestinal use (ATC A07AA), may also be considered for inclusion (Table 2.2).

Table 2.2 Groups of human antimicrobial agents that may be included in surveillance of antimicrobial use in humans

Groups of antimicrobial agents	ATC codes
Antimicrobial agents for intestinal use	A07AA
Antibacterials for systemic use	J01
Antifungals & Antimycotics for systemic use	J02; D01BA
Antimycobacterials	J04
Antivirals for systemic use	J05
Antimicrobial agents used as antiparasitic agents	P01AB
Antimalarials	P01B

In Europe, notable advances have been made in the surveillance of use of antimicrobials at both the country and at the European level. Surveillance of the use of antimicrobials in humans is coordinated by the European Centre for Disease Prevention and Control (ECDC), through the European Surveillance of Antimicrobial Consumption Network (ESAC-Net) for antimicrobial consumption and the Healthcare Associated Infection Network (HAI-Net) for antimicrobial use in acute care hospitals. ESAC-Net is a network of national surveillance systems that provides European reference data on antimicrobial consumption in the community and in hospitals.

2.2.1 Surveillance of national antimicrobial sales data

To collect and report national antimicrobial sales data, it is important to identify all distributors of antimicrobials in the country. Identification of all distributors is typically only possible if the medicine market is regulated in a manner that requires that all distributors be licensed by a competent authority. Conducting surveillance of national antimicrobial sales data is not a viable option in countries that do not have a regulatory framework that requires licensing of distributors of medicines.

Approaches to collecting national antimicrobial sales data will vary from country to country due to variations in the infrastructure of drug distribution systems and in the regulatory frameworks. A first step in conducting surveillance of national antimicrobial sales data is to describe the system of distribution of antimicrobials in the country, including identification of sales points outside the mainstream regulatory system (e.g. internet sales). It is vital to assess what each data source represents (e.g. data coverage) in order to identify the most appropriate points for data collection. A protocol detailing the data collection plan, and a data collection instrument, should be developed. ESAC-Net has established a standard approach for collection of data at the medicinal product level. To follow the ESAC-Net approach, a valid national register of available antimicrobials is needed. The ESAC-Net approach also describes the variables that should be collected for each antimicrobial.¹

¹ For the ESAC-Net surveillance of antimicrobial consumption report see: <http://ecdc.europa.eu/en/publications/Publications/antimicrobial-consumption-europe-esac-net-2012.pdf>



When conducting surveillance of national antimicrobial sales data, sales data should be reported on a regular basis. The national antimicrobial sales data should be reported according to the classes of antimicrobial agents of the Anatomical Therapeutic Chemical (ATC) classification system. Reports of national antimicrobial sales data should also report the appropriate population data (e.g. specification of the appropriate denominator). A preferred approach for reporting antimicrobial use data on a population level is to calculate defined daily doses per 1000 inhabitant-days; calculation of such a rate enables comparison of national antimicrobial sales data between regions and countries. An example of how national sales data can be reported is available in the interactive database from ESAC-Net.²

2.2.2 Point prevalence surveys on antimicrobial use in hospitals

Public policies and specific interventions to improve the quality of antibiotic use should be informed by the antimicrobial use surveillance data. The first step in improving the quality of antibiotic use is to establish the extent of inappropriate use of antibiotics. Meaningful comparisons of antibiotic use patterns can only be made between studies using similar study designs, definitions and data collection methods. The cross-sectional point prevalence survey design is useful in this regard because it is relatively simple to implement and can be structured to collect basic information on patients (antibiotic treatment, indication for treatment, the underlying disease).

Point prevalence surveys can provide useful data on patterns of hospital antimicrobial prescribing. Additionally, this survey would provide insight into the determinants of antimicrobial use. Data obtained from point prevalence surveys can be used to: 1) quantitatively and qualitatively compare antimicrobial use in different countries or regions; 2) identify targets for quality improvement such as adherence to hospital guidelines, documentation of antibiotic therapy, or perioperative prophylaxis; 3) help design hospital interventions aimed at promoting appropriate use of antimicrobials; and 4) assess the effectiveness of interventions (if surveys are repeated on a regular basis). Nationally, point prevalence surveys of hospitals should be conducted at least annually and be supported by a national surveillance network, if possible. Ideally, prevalence data should be collected routinely from hospitals (for example, four times a year) as part of an on-going monitoring programme.

For point prevalence surveys, data can be extracted from various sources, preferably written sources, such as patient records and computer databases. It is strongly recommended that pilot studies of patient-level data collection be conducted prior to the full point prevalence survey. A pilot study will help evaluate and refine the methodology by identifying problems in the survey design and data analysis and also providing an estimate of the workload per patient. Ideally, participating physicians should be asked to conduct a one-day cross-sectional hospital-based point prevalence survey, in which all included hospital wards are audited at once. The types of ward to be included should be predefined. The surveys should not take place on the weekend or on public holidays. Surgical wards should not be audited on the day after a weekend or public holiday, in order to capture information about prophylaxis in the previous twenty-four hours. Medical wards may be audited on any day of the week. In some countries, it may not be feasible to survey an entire hospital in one day. In this case, the survey can be conducted over several days, with a maximum of two weeks. In order to avoid duplicate records resulting from the movement of patients within the hospital, it is recommended that a whole ward should be

² For the ESAC-Net interactive database, see http://ecdc.europa.eu/en/healthtopics/antimicrobial_resistance/esac-net/database/Pages/database.aspx

surveyed on one day.

When organizing a point prevalence survey on a provincial or national level, a wide variety of hospitals with different ward and patient characteristics should be recruited to ensure that data are representative. In principle, efforts should be made to ensure that the sample of participating hospitals is representative of the larger (e.g. national) population. The sample should be random, possibly stratified by hospital type or size. Any interested hospital-based physician should be able to indicate his or her interest in the point prevalence survey by contacting a study coordinator. Participating physicians could be invited through national or local associations and conferences.

A point prevalence survey is best carried out by a multidisciplinary team of health professionals, including, where available, infectious disease specialists, infection control teams, clinical microbiologists, epidemiologists and clinical pharmacists. Members of the team should receive a detailed standardized protocol to ensure uniformity of data collection. An example of inclusion criteria for a point prevalence survey is the collection of detailed data from medical records for all inpatients (patient who stayed in hospital overnight) with active antimicrobial prescriptions at 8:00 AM on the day of the survey. Prescriptions after 8:00 AM on the day of the survey are excluded from the survey. Such a point prevalence survey would exclude day patients (didn't spend night in the hospital), outpatients and emergency admissions on the day of the survey. When conducting a point prevalence survey, it is important to calculate a denominator of the number of patients in the hospital. Examples of how to calculate a denominator for the above point prevalence survey include: total number of eligible inpatients at 8:00 AM on the ward surveyed or total number of eligible (occupied or empty) beds attributed to inpatients at 8:00 AM on the ward surveyed.

The following data should be collected in a point prevalence survey: the patient's age, sex, weight and ventilation status; the antimicrobial agent, single unit dose and number of prescribed doses per twenty-four hours; route of administration; anatomical site of infection; whether infection was acquired in the community or in hospital; details of prophylaxis for surgical patients (duration of prophylaxis such as one dose, one day, or greater than one day); and whether or not a diagnosis or indication for treatment was recorded in the notes when antimicrobial treatment was started. To facilitate data collection on reason for treatment, a predefined list of grouped items may be used. Co-morbidities may also be recorded. If all patients (including those not receiving antimicrobial treatment) are surveyed then the age, sex and possibly co-morbidities should be recorded. As a minimum, all patients receiving antibacterial drugs should be surveyed, with information on indication for use, dose and the patient's age and sex. After the survey, the prescribed antimicrobial products should be grouped according to the ATC classification; this will allow standardized reporting and comparison of results.

Large point prevalence surveys on antimicrobial use in hospitals have been, or continue to be, conducted. An example of an on-going large point prevalence survey is a worldwide survey of antimicrobial use in hospitals called GlobalPPS³ which is led by the Laboratory of Medical Microbiology of the University of Antwerp, Belgium. This group also runs GARPEC⁴ for antimicrobial use in children. In Europe and the United States of America (the USA), regional point prevalence surveys that gather information on both antimicrobial use and health-care associated infections are conducted at regular intervals^{5,6}. The principal indicators produced

3 <http://www.global-pps.org>

4 <http://www.garpec.org>

5 http://ecdc.europa.eu/en/healthtopics/Healthcare-associated_infections/point-prevalence-survey/Pages/Point-prevalence-survey.aspx

6 <https://www.cdc.gov/hai/eip/antibiotic-use.html>

by point prevalence surveys are prevalence rates for individual antibiotics, expressed as the number of treatments per 100 patients. If information about daily doses is collected, average prescribed doses can be computed. Other descriptive statistics, such as overall prevalence of antimicrobial use, and use by ward, infection site and antimicrobial class, can also be reported.

The uniformity of data collection and the data validation process in a point prevalence survey help guarantee a standardized, solid database for cross-sectional analyses. A point prevalence survey allows determinants of antibiotic use among inpatients to be investigated. The simplicity of the protocol makes the survey feasible, achievable and sustainable. It offers simple case definitions to aid auditing of antibiotic use, without recourse to complicated algorithms, such as diagnostic criteria. It further collects detailed data only on patients with an active antimicrobial prescription. This study design can simply measure drug use, or can be used for a criterion-based assessment of drug use in relation to guidelines or restrictions. If repeated regularly, such studies can contribute to sustained awareness of the need for careful use of antibiotics, and can be used to evaluate hospital-based interventions, such as the development of a local antimicrobial stewardship programme. The online data-entry and reporting tool offers the opportunity to include other data. Participants may be invited and encouraged to complete other questionnaires regarding current empiric antibiotic guidelines. The generalizability of the findings could be limited by the methodological approach. A one-day point prevalence survey in a small hospital, for example, would capture small numbers of patients with specific conditions. The point prevalence survey should therefore not be used for benchmarking. Nevertheless, antimicrobial use prevalence rates obtained through repeated point prevalence surveys seem to remain stable over time (8). The point prevalence survey does not collect information about the clinical justification and duration of antibiotic therapy, whether a suitable culture was obtained, whether the treatment is appropriate for the infection, or whether the surgical prophylaxis and its duration are justified. Other study designs (e.g. longitudinal studies) would be needed to collect such information.

2.2.3 Longitudinal surveys of antimicrobial use in hospitals or the community

Longitudinal studies can provide more detailed information on antimicrobial use than point prevalence surveys. For example, longitudinal studies allow both incidence and prevalence of antimicrobial use to be estimated. Additionally, longitudinal studies gather information about treatment duration and patient risk factors. Although longitudinal studies are time-consuming, they are often worthwhile because they give a clearer picture of what is happening at the patient level.

Longitudinal studies can be performed both in health institutions and in the community setting. They are easier to perform in health institutions, such as hospitals and nursing homes, where conditions can be more easily controlled than in the community. The studies may be prospective or retrospective (prescription databases or medical records). In addition to information about patients, indications and antimicrobial agents, longitudinal studies can also provide information about disease outcome, clinical presentation, laboratory results, and duration of treatment. Electronic prescribing has increased over the years. Consequently, databases now have full medical and prescribing information on a continuous basis at the individual level. Such databases are very powerful, and can address a range of issues, including reasons for changes in therapy, adverse effects and health outcomes.

Ideally, data for longitudinal studies should be collected routinely as part of an ongoing monitoring programme. Data may be collected continuously, over a defined period (e.g. part of daily work), or on a rotating basis (e.g. by time, disease, prophylaxis procedures, ward or type of patient). Nationally representative data should be collected annually, if possible. It is important

that the sample chosen for the longitudinal study is as valid and representative as possible. Epidemiological and statistical expertise is, therefore, desirable when designing longitudinal studies. Nevertheless, collecting accurate data may be a challenge so it is important to focus on the most essential information. Data collection may have to be limited in order to obtain maximum compliance.

Collection of end-user data on antimicrobial use can be challenging and there are a number of barriers to the acquisition of high quality, comprehensive data on antimicrobial use. Ideally, accurate, detailed data should be obtained from all persons using antimicrobials. Only a few countries have mandatory and automated reporting systems using sales data from pharmacies or prescription data from prescribers. In this setting, it should be remembered that information on sales and prescriptions does not necessarily reflect exact usage, since patient adherence is a confounding factor. Most countries do not have good registries or other sources of information on antimicrobial use, and periodic surveys are often needed.

When recruiting participants for a longitudinal study, it is rarely possible, due to logistic reasons or because of resource limitations, to include all eligible persons, institutions and general practitioners in a region or country. Therefore, some type of sampling is required. Efforts should be made to ensure that the sample of participants is representative of the larger population. As longitudinal studies are time-consuming, efforts should be made to motivate the participants continuously throughout the project, and to encourage them to comply with data collection throughout the study period. If the study is performed in institutions, only a few individuals should be responsible for data collection. When considering inclusion criteria, the examples of subject inclusion and exclusion given for point prevalence surveys also apply to longitudinal studies. In addition, in longitudinal studies, it is necessary to determine how many people received antimicrobial prescriptions over the entire course of the study period. Similarly, the denominator data in longitudinal studies are similar to those for point prevalence surveys. It is necessary to determine how many people were at risk of antimicrobial prescriptions over the entire course of the study period.

When considering data collection in a longitudinal study, it is often not feasible to include all antimicrobials and all types of infections in the study, therefore, a selection will have to be made. Criteria for prioritization could include severity or frequency of particular infections, importance of specific drugs for antimicrobial resistance, or general importance of certain antimicrobials (e.g. quinolones, third- and fourth-generation cephalosporins and macrolides). Such a selection allows data to be collected, for example, on antimicrobial use by type of infection or for the antibiotics that contribute most to the development of resistant bacteria. A detailed project protocol should always be developed, which includes the list of variables to be collected. A pilot study is recommended, and data collectors should be trained to ensure that the same procedure is followed.

The data to be collected in longitudinal studies are similar to those collected in point prevalence studies. However, longitudinal studies provide an opportunity to follow patients through their infection and their course of antibiotic therapy. As a minimum, data should be collected on the products used, the route of administration, the number of persons treated and the total number of persons at risk of exposure (located in the area at the time of study) during the study period. The duration of the study will depend on the resources available but should be long enough to provide sufficient data (e.g. three months for longitudinal cohort studies). Further information could include dose, duration of treatment, age and sex of the patient, and the purpose of administration (e.g. for prophylaxis or treatment of specific indication). Risk factors for use (e.g. the use of a catheter, immunomodulating treatment, and co-existing diseases, such as chronic obstructive pulmonary disease and cancer) are also important to an understanding of why and

for whom antimicrobials are used.

When deciding what additional information to collect, it is important to keep in mind the time that will be needed for data collection and the participants' ability to comply with correct data collection. Too exhaustive data collection may undermine the quality of data. Data on the general characteristics of the institution, physician-practice or area in question are also valuable (e.g. general housing or grouping information, types of patients or prescribers and demographic indicators).

When reporting the results of a longitudinal study, the standard indicators of antibiotic consumption in a hospital are defined as daily doses per 100 bed-days, or days on therapy per 100 bed-days. The most widely used indicator for outpatient antibiotic consumption is defined as daily doses per 1000 inhabitant-days. Other indicators that may be reported include appropriateness of treatment for the indication and appropriateness of the duration of therapy. Longitudinal studies of long duration may also be able to report temporal trends.

Properly conducted longitudinal studies provide much of the same data as point prevalence surveys. Together with detailed information on trends in consumption, they enable prospective audits of consumption of antimicrobial agents with direct interaction and feedback to prescribers. This strategy has proven effective in improving antimicrobial prescription practices and reducing costs. They can also investigate the clinical justification for antimicrobial therapy and its duration, and whether or not suitable ancillary tests (e.g. culture and sensitivity) were carried out. The limitations of longitudinal studies are related to their greater complexity, cost, and difficulty compared with point prevalence surveys.

2.3. Surveillance of use of antimicrobials in animals

The surveillance of antimicrobial use in animals is more complex than in humans due to the variation in use patterns by different animal species and production types (e.g. beef and dairy cattle). There are a variety of approaches for conducting surveillance of the use of antimicrobials in animals. Of particular note, OIE has published standards on monitoring the quantities and usage patterns of antimicrobial agents in animals. These approaches include surveillance of national antimicrobial sales data, and collecting data on consumption of antimicrobials by animal species. Surveillance of national antimicrobial sales data is relatively inexpensive to conduct and provides an overall picture of the quantities of antimicrobials used at the regional or national level. However, the regulatory framework or authorization for conducting surveillance of national antimicrobial sales data is not available in all countries. When national antimicrobial sales data are not available, alternative strategies for obtaining data on overall quantities of antimicrobials used for animal production can be applied. Furthermore, national sales data do not provide information on consumption of antimicrobials by animal species since most antimicrobials are used in several animal species. Approaches for collecting data on consumption of antimicrobials by animal species include continuous data collection (e.g. prescription data from pharmacies, veterinary clinics, or farms) and longitudinal studies at the farm level. Longitudinal studies at the farm level may also be helpful in deriving population consumption estimates at the animal species level, and such longitudinal studies can also include collection of complementary information on reasons antimicrobials are used such as indication or diagnosis.

When conducting surveillance of the use of antimicrobials in animals, it is important to ensure that information is kept confidential. For example, when conducting farm surveys, participants need to be assured that individual farms will not be revealed in any internal or external report.



Programmes monitoring antimicrobial use in animals should include antimicrobial agents classified in the ATC classification system for veterinary medicinal products (ATC-vet) as antimicrobials for intestinal use (QA07AA; QA07AB), antimicrobials for intrauterine use (QG01AA; QG01AE; QG01BA; QG01BE; QG51AA; QG51AG), antimicrobials for systemic use (QJ01), antimicrobials for intramammary use (QJ51), and antimicrobials for antiparasitic use (QP51AG). See Table 2.3. Antimicrobial growth promoters, which are not included in the ATC-vet system, should also be included.

Table 2.3. Groups of veterinary antimicrobial agents that may be included in surveillance of antimicrobial use in animals

Antimicrobial agent group	ATCvet codes
Antimicrobial agents for intestinal use	QA07AA; QA07AB
Antimicrobial agents for intrauterine use	QG01AA; QG01AE; QG01BA; QG01BE; QG51AA; QG51AG
Antimicrobial agents for systemic use	QJ01
Antimicrobial agents for intramammary use	QJ51
Antimicrobial agents used as antiparasitic agents	QP51AG

In Europe, notable advances have been made in the surveillance of use of antimicrobials in animals in several countries. Surveillance of antimicrobial use in animals in Europe is coordinated by the European Medicines Agency, through the European Surveillance of Veterinary Antimicrobial Consumption (ESVAC). Currently, ESVAC collects information on overall national sales of veterinary antimicrobial agents across the European Union. Other countries, such as Canada and the USA, also collect overall sales data on veterinary antimicrobial agents, including antimicrobial growth promoters. The Canadian Integrated Programme for Antimicrobial Resistance Surveillance (CIPARS) reports annually on consumption of antimicrobials used in animals in Canada. The Canadian Animal Health Institute, a veterinary pharmaceutical industry association, provides the aggregated data (9). In the United States, the Food and Drug Administration (FDA) provides annual reports of the quantities of antimicrobials sold or distributed for use in food-producing animals. The pharmaceutical industry is required by law to provide this information to the FDA on an annual basis (10).

2.3.1 Surveillance of national antimicrobial sales data

To obtain optimal coverage of data on antimicrobials sold it is important to identify all distributors in the country or region in question. This would typically be possible only if the medicine market is regulated in a manner that requires all distributors have a license provided by a competent authority. Unless this is the case, surveillance of antimicrobial sales data should not be the selected approach for conducting surveillance of antimicrobial use in animals.

Approaches to collecting data on sales of antimicrobials in animals will vary from country to country, because of variations in the infrastructure of drug distribution systems. As a first step, the system of distribution of antimicrobial agents to animals within a country should be identified. Each data source should be assessed in order to identify the most appropriate points of data collection (e.g. the data sources that can provide optimal coverage of the data). Also, sales points outside the ordinary distribution system should be identified and assessed (e.g. internet sales, import of medicated animal feeds, and movement of antimicrobial agents across borders).

When conducting surveillance of national sales of antimicrobials in animals, a protocol on the collection of data should be developed. This protocol should include population data and be adapted to the local situation. A template for the collection of data should be developed and tested. OIE has published a protocol for collection of sales data from the OIE member states⁷. The OIE template for submitting data to OIE can be found in Annex V of the OIE protocol. The European Medicines Agency (EMA) has also published a protocol and data collection template for collecting sales of veterinary antimicrobial agents at the national level (11); this protocol and form is used by countries participating in ESVAC. The ESVAC collected veterinary medicine data at both the products level and packages sold level. OIE has harmonized their data collection protocol and template to that used in ESVAC. The ESVAC protocol and template present the variables that should be collected for each antimicrobial in order to enable calculation of the amount of active ingredients of antimicrobials sold (11).

To facilitate comparison of antimicrobial sales data across years and among countries, it is important to consider the size of animal populations at the national level. Data sources used to obtain animal demographic data for the analysis of sales data should be publicly available. Such animal population data can be used to create rates of antimicrobial use by animal species. When calculating such rates, it is important that the numerator only include antimicrobials used in that animal species and the denominator only measure the size of the animal species. In Europe, the denominator used by ESVAC is the population correction unit (PCU), which is an estimate of the combined weight of livestock and slaughtered animals in the country. The PCU takes into account the animal weight at the time that treatment was most likely given. It also takes into account that animals transported for slaughter or fattening in another country are likely to have been treated in the country of origin (12). ESVAC has developed a template for collecting data on the various animal categories included in the denominator for sales data. It also includes the calculation of the PCU⁸.

When reporting national antimicrobial sales data, the overall national sales data should reflect the quantity of antimicrobial agents (e.g. mg of active substance) sold per year (or other unit of time). Sales data of antimicrobials used for therapeutic and growth promoter purposes should be presented separately, if possible. In order to harmonize the national sales data reporting across countries, the sales data should be reported according to the classes defined by the ATC-vet system. Of note, some antimicrobial growth promoters are not included in the ATC-vet system since the ATC-vet system is limited to antimicrobials used for therapeutic purposes. It is recommended that antimicrobial growth promoters that are not included in the ATC-vet system be reported by analogous antimicrobials that are defined by ATC (13).

When ESVAC reports national antimicrobial sales data, the indicator used to report overall sales data is mg active substance per PCU (mg/PCU), where 1 PCU = 1 kg of different categories of livestock and slaughtered animals (14). CIPARS also reports overall sales using mg/PCU. Data on overall sales can be split into products intended for companion animals and those generally used for food-producing animals and horses. This data is based on information in the marketing authorization, formulation, and strength. Data can be reported in terms of group (herd) treatment and treatment of individual animals.

2.3.2 Surveillance of antimicrobial consumption by animal species

Data on quantities of antimicrobials used by animal species can be obtained from a variety of

7 http://www.oie.int/fileadmin/home/eng/International_Standard_Setting/docs/pdf/SCAD/A_SCAD_Sept2015.pdf

8 http://www.ema.europa.eu/docs/en_GB/document_library/Template_or_form/2013/10/WC5001523_12.xls

sources such as prescriptions, veterinary practice records, health records, treatment log books, delivery notes and invoices. The data can be collected using a census model, involving all or most farms of the species included in the surveillance. This census model would typically involve continuous collection of data. For most countries, such systems cannot be established due to cost and other factors. For these countries, collection of data on a sample of farms can be used. If a sample of farms is used, the sample should be representative of the national population of the animal species under surveillance. Another approach for estimating the quantities of antimicrobials consumed by animal species is by stratification of national sales data by animal species.

Ideally, data on antimicrobial consumption by animal species should be collected routinely as part of an on-going surveillance programme. Data on antimicrobial consumption by animal species can be collected continuously (e.g. for each species each year, or on a rotating basis by species). An important use of continuously collected data at the farm and species level is the comparison of antimicrobial consumption at farmer or veterinarian level against a standard. This approach is called benchmarking and has been used in some countries including Denmark and the Netherlands.

Prior to embarking on a survey or study to estimate antimicrobial consumption by animal species, consideration should be given to conducting a pilot to evaluate and refine the methodologies (e.g. data collection instruments and validation mechanisms). Furthermore, a protocol and a data collection template should be developed. It is very important to take into account how the data collected will be managed.

2.3.3 Continuous collection of consumption data by animal species

Continuous collection of data on consumption of antimicrobials at the farm level from farmers' records, veterinarians' records, prescriptions, or delivery notes requires automated recording of standardised data that are electronically stored and subsequently transferred to a database. Establishing continuous electronic data collection typically requires that the antimicrobials under surveillance are prescription only and that use is electronically recorded. For governmental surveillance programmes that collect data from veterinarians and distributors (e.g. wholesalers, pharmacies) on antimicrobials, all consumption for all or almost all species should be included to avoid selection bias. This also allows for validation of the data against overall national statistics. When data are collected by the food animal industry, such as in the Netherlands, the animal species would be the major species and production categories of concern to that industry.

In Denmark, a programme entitled "VetStat" was established by the government in 2000. VetStat consists of standardized data from dispensed prescriptions from pharmacies and feed mills that are electronically recorded and reported; this includes sales of antimicrobials for use in veterinary practices. Veterinarians use electronic journal software systems and these software systems are designed to automatically transfer data on all treatments regarding production animals to VetStat in connection with billing. In the Netherlands, data on consumption of antimicrobials by animal species is collected by the private livestock sector⁹. Standardized information on veterinarian prescriptions and sales of medicines to the farmers is entered into Practice Management Systems (PMSs) and transferred to a central database that is part of integral quality assurance systems. Most of the data transfers take place through VetCIS (www.vetcis.nl), a data hub system set up by a joint collaboration between the Royal Dutch Veterinary Association, the main veterinary drug wholesaler in the Netherlands (AUV), and the association

⁹ <http://www.autoriteitdiergeneesmiddelen.nl/en/home>

of the veterinary pharmaceutical industry in the Netherlands. Data in VetCIS is subsequently transferred to the private sector databases. Some of the data are directly transferred from the PMSs to the private sector databases or the data are entered by veterinarians through internet portals of the sector systems.

2.3.4 Collection of data from a sample of farms

In most countries, antimicrobial prescription data are not routinely collected or reported from the farm level. Therefore, an alternative strategy of collecting information on consumption of antimicrobials is required. In Canada, for example, CIPARS farm-level surveillance of antimicrobial use in grower-finisher pigs and broiler chickens utilizes a sentinel veterinarian and farm approach to estimate prevalence of antimicrobial use at the farm level. Veterinarians are recruited to identify typical farms, and to collect antimicrobial use information through review of antimicrobial use records and the administration of questionnaires to farmers. The collected antimicrobial use data apply to a specific cohort of grower-finisher pigs or flock of broiler chickens. Faecal samples are also collected for the purposes of antimicrobial resistance surveillance at the farm level. In the United States, the National Animal Health Monitoring System (NAHMS) conducts periodic national on-farm studies in specific food animal species. Farm-level antimicrobial use information is obtained through the administration of questionnaires to random samples of farms that are nationally representative. Farmers can be useful sources of data on consumption of antimicrobial agents by animal species, production type and age class. In some countries, farmers are required to maintain records of treatment, which can be a valuable source of data. In other countries, however, this is not the case and it is often not feasible to set up an independent system for collecting data on consumption from all farms. Therefore, it may be necessary to carry out data collection on a subset or a sample of farms. A first step would then be to identify the structure of the industry (e.g. pig farms in terms of the number of farrow-to-finish, farrow-to-weaner, and/or weaner-to-finisher farms). Ideally, the sample of farms is representative of the national population of the animal species under surveillance. In order to ensure that the sample selected for study is as valid and representative as possible, epidemiological and statistical experts must provide input into the design of the programme. There are many challenges to collecting accurate data so it is important to focus on the most essential information. Data collection may need to be limited in order to reduce the demands on the veterinarian or farmer and to obtain maximum compliance.

There are several potential barriers to the acquisition of high quality, comprehensive data on antimicrobial consumption from farms. In most countries, end-users do not routinely keep detailed and up-to-date records that are useful for estimation of drug use. Thus, periodic surveys involving the use of questionnaires or other tools are often needed. Most farmers are not trained in veterinary medicine or pharmacology, and many do not clearly distinguish among various types of medication. Consequently, it is often difficult to obtain much more than product label data. The interviewer will need to collect additional information in order to determine the identity of antimicrobial agents of interest. Except on very small farms, farmers frequently do not know precisely how many animals are on the premises at any one time, or how they are distributed by production type (e.g. cows, calves, heifers, fattening cattle) so it may be necessary to rely on estimates.

In most countries, many species of animals are kept for food production, transportation or companionship. It is often not feasible to monitor all species every year. Therefore, countries will need to prioritize certain species (e.g. cattle) and production types (e.g. beef, veal or dairy). In assigning priority, the estimates of the size of the animal population, preliminary data on consumption of antimicrobial agents by species, species-specific rates of carriage of important foodborne pathogens, and other factors that could contribute to the exposure of humans to



resistant bacteria should be assessed. Typically, priority should be given to the animal species and production types that are most important to food production, are suspected to have the highest rates of exposure to antimicrobial agents, and are also known sources of resistant bacteria for humans.

It is rarely possible, due to logistic reasons or resource limitations, to include all farmers in a region or country. Some type of sampling is therefore required. Efforts should be made to ensure that the sample of participating farms is representative of the larger population. If an inventory of farms exists, it should be used as a basis for probability-based sampling (for a given region, selection of a random sample, stratified by farm size for a given species). In most countries, it will be difficult or impossible to obtain registries of farms that can be used for this purpose. Alternative ways of selecting participants, such as non-probability sampling, will be necessary. Options include asking practising veterinarians to identify farms, or soliciting volunteers through notices in trade magazines or abattoirs. It needs to be recognized that such non-probability sampling of farms may produce biased estimates. Sampling of farmers should be stratified on the basis of the animal species of concern; consideration should also be given to animal type (e.g. beef or dairy), production type (e.g. intensive or extensive), and farm size (e.g. in terms of number of animals). Incentives for participation (e.g. financial remuneration) may be useful but can result in substantial programme costs. There are obvious advantages to recruiting farmers who maintain good quality records of antimicrobial treatments, as well as animal inventories and records of the dates when animals enter and leave the herd. This latter information is needed for calculation of treatment rates, etc. While recruiting farms that maintain good records is useful, the degree to which these farms are representative of the overall animal production in the country or region should also be considered.

When conducting an antimicrobial consumption survey of a sample of farms, sources of antimicrobial consumption data include health records, treatment log books, delivery notes, invoices, veterinary prescriptions, veterinary practice records, and interviews of farmers. After thoughtful consideration of the options available, a decision on the appropriate data source or combination of data sources should be made before conducting data collection. To reduce selection bias, data on all antimicrobials prescribed and administered should be collected; however, it may be prudent to exclude some formulations of antimicrobials (e.g. dermatological preparations such as cutaneous sprays) because it is difficult to establish defined daily doses for such products. Additionally, the contribution of such products to overall consumption is typically very low.

As a minimum, the following data should be collected at the farm level for the period of interest (e.g. day of the survey, production cycle of flock, cohort of grower-finisher pigs):

- number of treated animals on the farm, by species, age, stage of production and weight in kilograms;
- names of antimicrobial product(s) used for treatment;
- name of the supplier of the product;
- dose;
- dosing interval (per day);
- number of days of treatment;
- route of administration;
- individual or herd treatment;
- reason for antimicrobial administration; and,
- number of food-producing animals on the farm by species, age, age class and weight.

This information is required to determine amounts of active ingredient, the frequency, dose and duration of administration of antimicrobial agents, and to calculate the prevalence of treatment.



Efforts should be made to record demographic data of the animal population at risk for treatment on the farm. This normally requires collection of data on the general characteristics of the farm (e.g. all livestock on the premises, all livestock owned by the farmer but located on other properties), species, age classes (e.g. piglets, sows, weaner pigs, finishing pigs) and general housing and grouping information (e.g. cows and calves on pasture, broilers in confinement in one barn). Basic data can be collected from treatment records or through questionnaires. Where possible, in order to avoid extra work for farmers and to minimize recall bias, data should be collected from existing records, which may include electronic or written farm records or on-farm quality assurance programme records. In most cases, however, some additional input from the farmer or a farmworker is required, and this can be a major obstacle to the collection of accurate and representative data. Considerable planning is needed to focus on collecting the most important data, using the methods that are simplest and quickest for the participants, in order to increase the likelihood of obtaining accurate and complete information.

Questionnaires have the advantages of being relatively simple for the farmer and entailing low costs for administration. They provide data pertaining mainly to treatment prevalence (e.g. the proportion of animals administered a course of treatment during a specified time period) and qualitative data on use (e.g. whether or not a specific antimicrobial agent was used on the study farm during the specified time period and the route of administration). The farmer may fill in the survey form personally, by hand or electronically. Alternatively, a member of the survey team may conduct an interview by telephone or during a farm visit. Visits are likely to produce more complete information and allow some of the data to be validated, for instance by inspection of facilities, drug storage cabinets and refrigerators. Questionnaires are useful for collection of point prevalence data, such as the number of animals treated the previous day, and information on routine or general treatment practices, farm characteristics and management practices. Collection of data that vary over time, for example, therapeutic treatment of individual animals, should be completed during an appropriate and efficient time interval such as the day of or the week before completion of the questionnaire. If farms have existing records (e.g. records required by law or for industry quality assurance programmes or farm production records) that contain the desired data, they can ideally be uploaded directly. They also can be used by a member of the survey team to complete the questionnaire, thus saving the farmer time and effort. Informal records, such as bills for medicated feed, may also be useful sources of data.

2.3.5 Stratification of sales data

National sales data can also be stratified to provide estimates of antimicrobial consumption by animal species. Beginning in 2016, drug sponsors in the United States are required by the FDA to provide estimates of sales stratified by major food producing species (cattle, swine, chickens and turkeys) in addition to figures on their overall sales of antimicrobials for use in food-producing animals¹⁰. Similar requirements are being proposed in Canada¹¹.

When reporting antimicrobial consumption data at the species level, species-level data should be reported in a standardized fashion that takes into account the number of animals treated over the reporting period or a unit similar to PCU. While at the international level, defined daily doses for humans have been assigned to antimicrobial agents for use in standardized reporting, an equivalent measure for animals has not yet been agreed upon internationally. Some countries have adopted the defined daily dose animals (DDDA) (15). Reporting may also include the duration of treatment by use of defined course dose animal (DCDA).

10 <http://www.federalregister.gov/articles/2016/05/11/2016-11082/antimicrobial-animal-drug-sales-and-distribution-reporting>

11 <http://www.gazette.gc.ca/rp-pr/p1/2016/2016-07-02/html/reg2-eng.php>

Which indicators to be used to report the consumption by animal species depends on the level of reporting. For reporting overall consumption for an animal species and year, the following indicator can be used: mg active substance/number of pigs produced/year or number of DDDA (per kg)/number of pigs produced/year. For reporting data by age group, the following indicator may be used: numbers of DDDA (for one kg)/the estimated live biomass in the age group/ the total population under surveillance¹². Examples of indicators that may be used to report farm-level antimicrobial consumption include: percentage of farms using a specific antimicrobial; median days of exposure through feed or water; quantity of antimicrobial used in feed by reason of use; percentage of broiler feed rations medicated with antimicrobial; and percentage of feedlots that treated cattle as a group with an injectable antimicrobial (metaphylaxis).

Recently defined daily dose for veterinary medicines (DDDvet) and defined course dose veterinary medicines (DCDvet) have been published for antimicrobials by the EMA for cattle, pigs and broilers. DDDvet and DCDvet are not applicable for antimicrobial growth promoters although DDDvet are used for growth promoters in Canada¹³. EMA's draft vision and strategy 2016-2020 on ESVAC outlines the vision to transfer DDDvet and DCDvet system to an international body such as the WHO Collaborating Centre for Drug Statistic Methodology. This action will make use of the DDDvet and DCDvet system at the global level¹⁴.

2.4. Data management to support surveillance of antimicrobial use

Documenting the quantities of antimicrobials used often requires several approaches (e.g. paper or electronic records) and multiple data sources (e.g. prescriptions, invoices, physician records, hospital records, or veterinarian records). As a result, data on antimicrobial use may vary greatly in granularity (individual pills, patient prescriptions, or aggregate statistics), type (antimicrobials purchased, dispensed, or administered) and antimicrobial use scenario (therapeutic, prophylactic, or growth promotion). There is also a wide range of potentially useful additional information relevant to understanding the decision to use a particular antimicrobial, such as clinical diagnosis, supportive diagnostic test results, patient expectations and financial considerations. Consequently, database design and needs for data management, analysis, and presentation can be very different from project to project.

There are two primary and complementary strategies that can be used to track antimicrobial use: quantitative and qualitative strategies. The quantitative strategy (quantity of antimicrobials used) is valuable for tracking the total antimicrobial use in different populations and over time. The qualitative strategy (why and how antimicrobials are used) is valuable for understanding the factors that contribute to the decision to use an antimicrobial and the appropriateness of such use. Both approaches can be applied to monitor antimicrobial use within health care facilities, the community, and on farms. Both quantitative and qualitative approaches have been successfully used to monitor the impact of educational and regulatory interventions on the use of antimicrobials.

12 www.danmap.org/

13 http://ema.europa.eu/ema/index.jsp?curl=pages/regulation/general/general_content_001493.jsp&mid

14 http://www.ema.europa.eu/docs/en_GB/document_library/Regulatory_and_procedural_guideline/2016/04/WC500204522.pdf

2.4.1 Quantitative antimicrobial use

This approach attempts to track total quantities of antimicrobials used at the local, regional, or national level. Depending on the data sources available, quantities may be expressed in terms of economic cost, total weight, defined daily doses, days of treatment, or other measures of antimicrobial use. In some instances, a quantitative antimicrobial use database may contain information at the patient or animal level, such as number of pills dispensed or prescribed. From such granular details, aggregate statistics can be calculated. In other cases, the only data available may be aggregate statistics, such as the number of packages of a particular antibiotic purchased by a health clinic in a given period. For surveillance of quantitative antimicrobial use in humans and animals, recommended data fields in the database include:

- sample population: country, year, animal species (if available);
- period covered (year, quarter, month);
- identity of antimicrobial: medicinal product identifier code, name or label;
- active substance: name, ATC code, ATC defined daily dose;
- package content: quantity (including quantity of active ingredients), units of measurement of active ingredients, number of items per package and, where relevant, conversion factor for associated salts and prodrugs;
- administration: pharmaceutical form, route of administration;
- consumption: number of packages used (sold, prescribed, reimbursed, delivered), duration of treatment.

Statistics derived from the above data include 1) number of kilograms of drug used; 2) number of defined daily doses; and 3) number of days of treatment.

2.4.2 Qualitative antimicrobial use

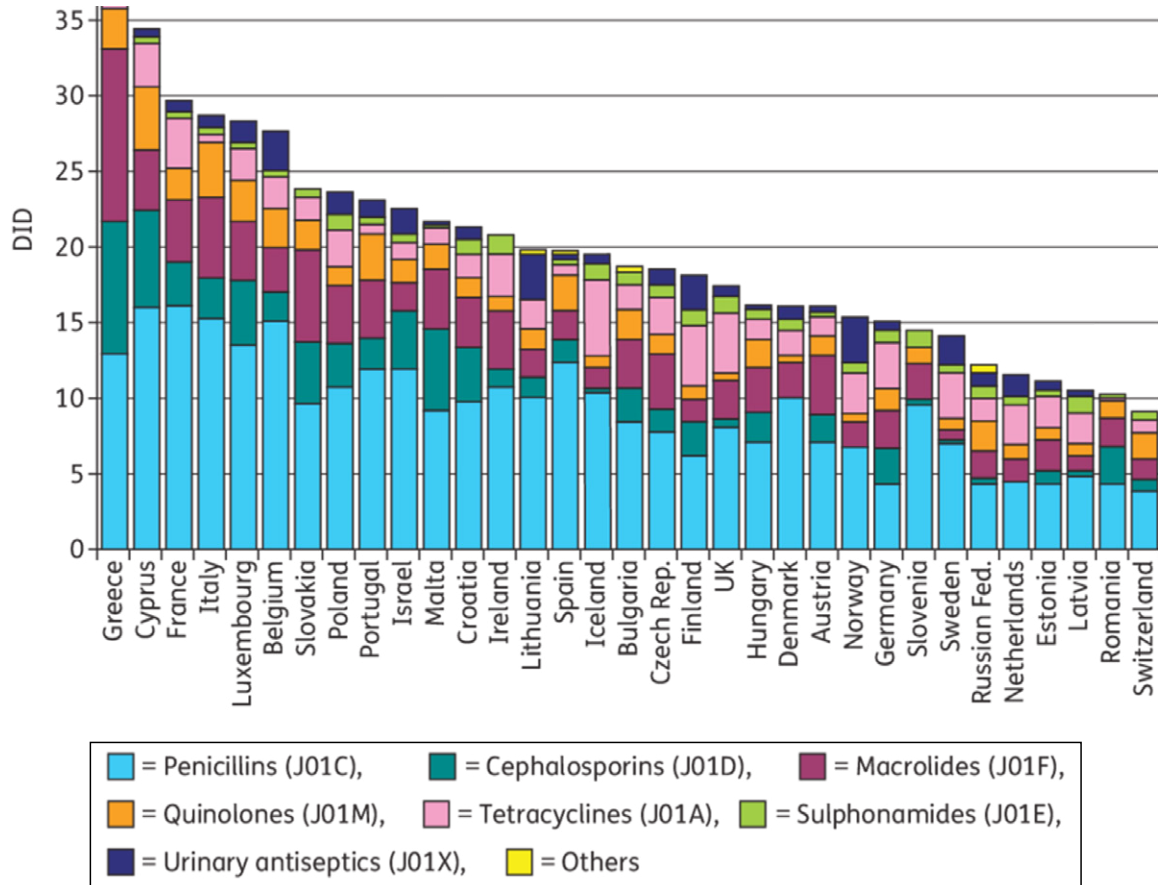
Understanding how and why antimicrobials are used is a more complicated issue than estimating the amount used. Despite this complexity, it is often simpler and more feasible to collect qualitative survey “snapshots” of antimicrobial use. Often, aggregate data on antimicrobial use may not be available to public health authorities by insurance systems or commercial entities, such as pharmaceutical companies and food producers. A useful series of documents has been developed and validated over time by WHO in collaboration with many international partners, to help guide the collection of data on antimicrobial use in a variety of clinical and non-clinical settings (16-19). Drug use indicators have proven to be a simple but valuable tool for highlighting deficiencies and prioritizing interventions in drug procurement, compliance with standard treatment guidelines, and the education of health care workers.

In collaboration with a number of partners, including the International Network for Rational Use of Drugs (INRUD), WHO has for many years supported drug use surveys in a variety of clinical and non-clinical settings, especially in low-resource countries. Some of the best models have been pioneered through European initiatives, most notably ESAC-Net and ESVAC. ESAC-Net was established in 2001 and collected aggregate statistics on the use of antimicrobials in the participating countries. From 2001 to 2011, the project included 35 countries. In 2011, the surveillance programme was transferred to ECDC. On an annual basis, each participating country collates aggregate statistics (reimbursement data or sales data) on the national consumption of antimicrobials from a variety of databases. Consumption is expressed in terms of number of packages or, if not available, as number of defined daily doses at the substance level. Separate data are presented for antimicrobial use in hospitals and in community settings. Significant efforts have been made to standardize protocols, definitions and data types. For most countries, the statistics reflect the amount of antimicrobials purchased or reimbursed.

Departures from the recommended protocol are described in the annual reports. Results are available to the general public on the ECDC website¹⁵. Fig.2.1 shows the significant differences

in patterns of antimicrobial use across Europe, both in total volume and in the distribution by antimicrobial class. Fig.2.2 shows similar data for penicillin. Fig.2.3 shows the annual variation in outpatient antimicrobial use. Fig.2.4 compares defined daily doses per 100,000 inhabitants for 21 European countries.

Fig.2.1 Consumption of antimicrobials for systemic use (ATC group J01) at ATC level 3 in the community, EU/EAA countries, 2012, expressed in DDD per 1000 inhabitant per day



Source: Johnson AP, Reeves DS. European Surveillance of Antimicrobial Consumption (ESAC): outpatient antibiotic consumption (1997-2009). J. Antimicrob. Chemother. 2011; 66(Suppl 6): 1-94.

Fig.2.2 Use of narrow-spectrum penicillins (J01CE) in humans as percentage of total antimicrobial consumption in Europe.

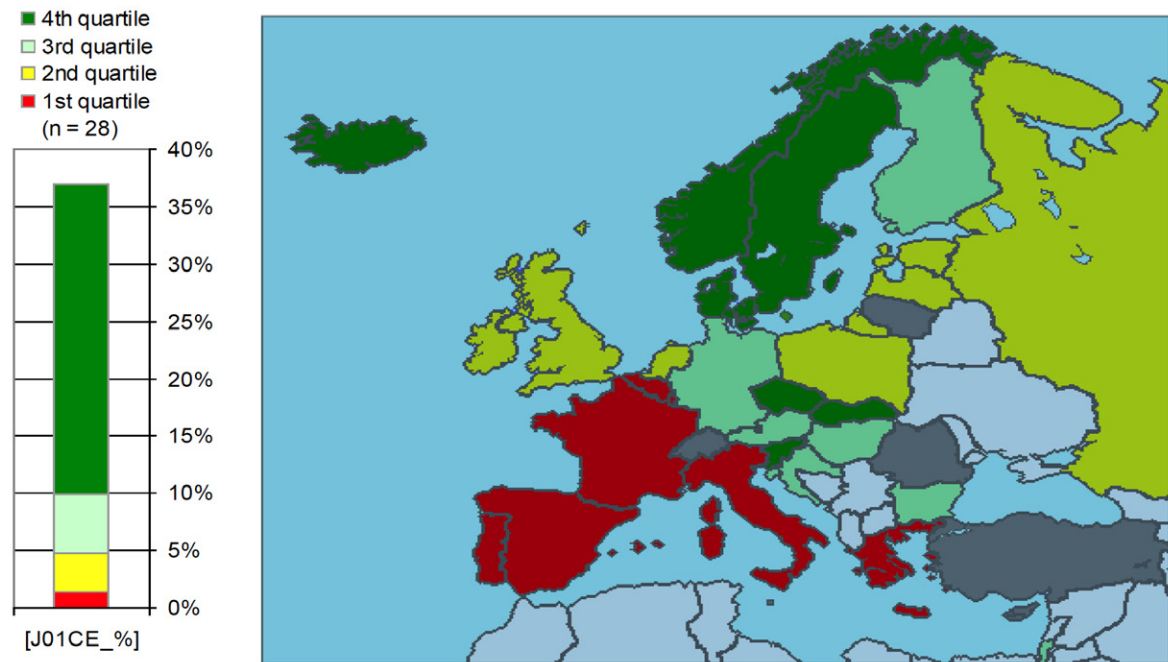
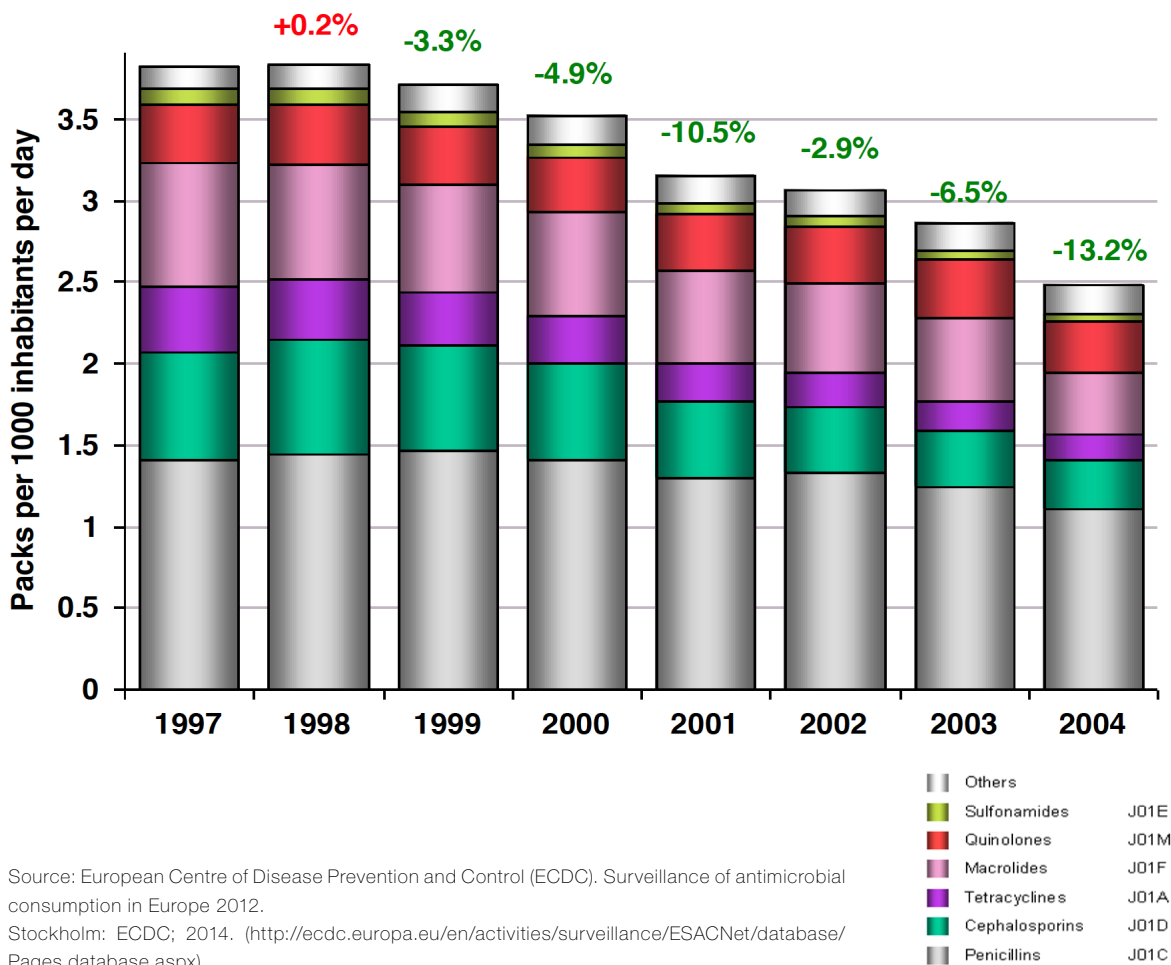
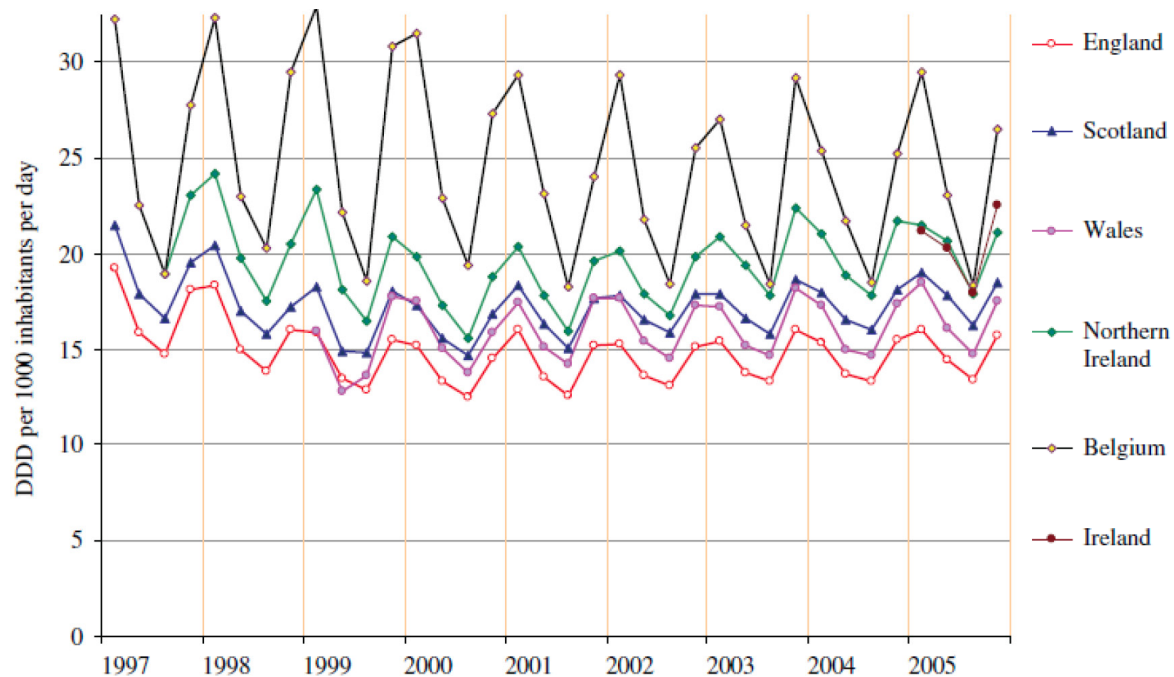


Fig.2.3 Change in outpatient antimicrobial use in Belgium, 1997-2004.



Source: European Centre of Disease Prevention and Control (ECDC). Surveillance of antimicrobial consumption in Europe 2012. Stockholm: ECDC; 2014. (<http://ecdc.europa.eu/en/activities/surveillance/ESACNet/database/Pages.database.aspx>).

Fig.2.4 Seasonal variation in outpatient ciprofloxacin use, expressed in DDD/1000 inhabitants per day, in the United Kingdom and Belgium, 1997-2005



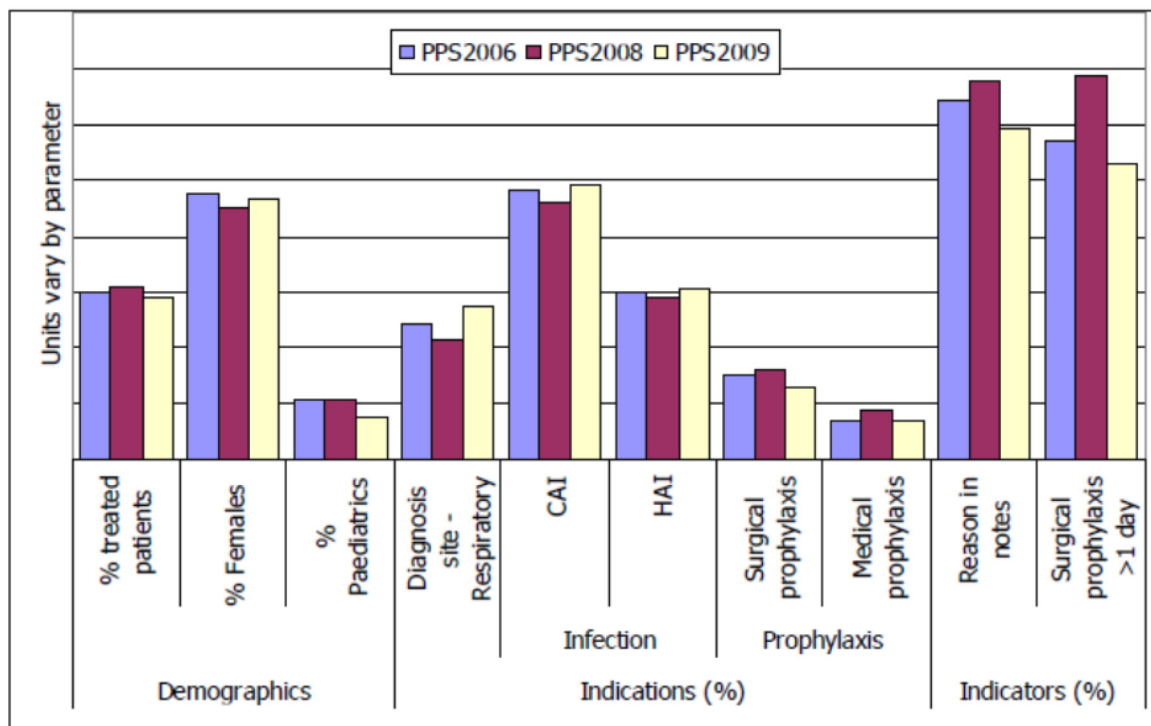
Source: Peter Davey et. al Outpatient antibiotic use in the four administrations of the U.K.: cross-sectional and longitudinal analysis. *Journal of Antimicrobial Chemotherapy* (2008) 62, 1441-1447.

The ESVAC project, run by the European Medicines Agency, has developed a similar protocol for the collection of aggregate statistics on sales of antimicrobials intended for animals. In 2011, ESVAC collected retrospective data from nine European countries that had comparable surveillance systems in place (20). In October 2016, ESVAC released its most recent report on sales of veterinary antimicrobial agents in 19 EU/EEA countries.

In 2005, the World Health Assembly adopted a resolution entitled “Antimicrobial resistance: a threat to global health security. Rational use of medicines by prescribers and patients” (WHA resolution A58/14)¹⁶. In support of this resolution, WHO assembled an extensive list of surveys of antimicrobial use in patients from all regions of the world (21). Results of indicator studies were published in a number of WHO documents (22,23). An example of regionally coordinated antimicrobial use survey is the ESAC-Net led hospital point prevalence survey which was initiated in 2006 as a pilot project with 20 hospitals in 20 countries. By 2009, 172 hospitals had submitted point prevalence surveys (Fig.2.5). In 2010, the ESAC point prevalence survey protocol was adapted and merged with an ECDC protocol for surveying health care-associated infections.

16 http://apps.who.int/gb/archive/pdf_files/WHA58/A58_14-en.pdf

Fig.2.5 Distribution of percentage of parenteral therapies among hospitals in the three EASC PPS.



Source: ESAC Yearbook 2009.

The data management needs for antimicrobial use programmes are more diverse than those for antimicrobial resistance studies, reflecting the variety of data sources and antimicrobial use settings and indications. Consequently, there is no single software that can handle all these needs, and many initiatives rely on locally developed or customized data solutions. Nevertheless, the growing acceptance of certain models for monitoring programmes should facilitate the development of new software tailored to these standardized protocols.

An early freely available tool was ABC Calc developed at the end of the 1990s. Many investigators successfully used the ABC Calc software for monitoring aggregate use statistics at the ward, facility, or national level. The latest distribution version of ABC Calc was implemented within Microsoft Excel, and relied on predefined formulas and reference values such as ATC classification and defined daily dose definitions. With ABC Calc, the user indicated the name of the pharmaceutical product and product details, including the active ingredient and, if relevant, the administration route, the amount of active ingredient per unit, the number of units per package, and the total number of packages purchased or consumed. ABC Calc then automatically generated the total number of kilograms of antibiotic used and, when definitions existed, the number of defined daily doses. Unfortunately, ABC Calc development was halted at the end of the 2000s due to mainly issues with maintenance.

A new software named AMC Tool was developed to supersede ABC Calc. AMC Tool is a standalone application that can be downloaded from the internet (<http://amu-tools.org/amctool>). AMC Tool has the same principles as its predecessor but it has extended functionalities such as additional ATC sub-groups (antimycotics, antifungals, drugs against tuberculosis, and antivirals), data entry, and antimicrobial consumption indicators. In addition to manual data

entry, AMC Tool can load pre-defined comma separated files to speed up the process of data entry. During data entry, AMC Tool validates the data entered by the user and will report entry error if present. The programme calculates aggregate statistics on antimicrobial consumption expressed in defined daily doses (DDD). There is also the possibility to report antimicrobial consumption by hospital activity (DDD/100 bed-days), but also to report by the population (DDD/1000 inhabitants/day). AMC Tool can export antimicrobial consumption data into a comma separated file. With AMC Tool, the user enters information about the name of product, package size (number of unit doses per package), strength (grams per unit dose), ATC substance code, route of administration, and the number of packages. The antimicrobial consumption is provided in defined daily doses aggregated along the different levels of the ATC classification. The user enters information about the activities (either hospital or community denominator) and the consumption is reported accordingly (either DDD/100 bed-days or DDD/1000 inhabitants/day).

The Global Point Prevalence Survey of Antimicrobial Consumption and Resistance (GLOBAL-PPS) is an ambitious project that was developed following experiences of three point-prevalence surveys carried out by the European Surveillance of Antimicrobial Consumption project between 2006 and 2009. The GLOBAL-PPS established a global network of point prevalence surveys and aims to include as many hospitals from as many countries from all continents. The Global-PPS was piloted in November 2014. The first full point prevalence survey was conducted from February-June 2015 and included 335 hospitals from 53 countries worldwide. It created global awareness about antibiotic use and resistance which is instrumental in planning and supporting national and local stewardship interventions in a range of resource and geographical settings. The evaluation of antimicrobial prescribing practices in hospitals and the identification of targets for quality improvement of antimicrobial prescribing were among the critical benefits for these hospitals. Further, the web based tool was easy to use, required minimal training for data-entry and analysis, and provided rapid feedback. Through repeated point prevalence surveys, this tool will enhance changes in practise and provide a way to measure the impact of interventions. Protocols, feedback reports that hospitals receive after data validation, and information on dissemination activities (ECCMID-posters) were presented at the 2016 ECCMID-congress in Amsterdam. The next Global-PPS is projected to be in 2017.

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3

Combined Analysis and Reporting



3. Combined analysis and reporting of a programme of integrated surveillance of antimicrobial resistance in foodborne bacteria

Combined analysis and reporting, whereby relevant information about the microbiological and epidemiological data from antimicrobial susceptibility testing and antimicrobial use are analyzed and reported together, should be a goal for all countries. To achieve combined analysis and reporting, a One Health approach is needed during the development, implementation, reporting and analysis of the antimicrobial resistance and antimicrobial use surveillance. Few countries have combined analysis and reporting of a programme of integrated surveillance of antimicrobial resistance of foodborne bacteria that includes surveillance of antimicrobial use and antimicrobial resistance across all sectors (food-producing animals, retail foods, and humans). In this final chapter, combined analysis and reporting of programmes of integrated surveillance of antimicrobial resistance in foodborne bacteria is described, selected examples are presented, and suggested steps for progressing toward combined analysis and reporting are offered. In addition, reporting options including risk communication, and suggestions for a step-wise approach towards implementation of an programme of integrated surveillance of antimicrobial resistance in foodborne bacteria is discussed.

3.1. Description of combined analysis and reporting of a programme of integrated surveillance of antimicrobial resistance in foodborne bacteria

Combined analysis and reporting of a programme of integrated surveillance of antimicrobial resistance in foodborne bacteria comprises the bringing together of antimicrobial use and antimicrobial resistance data across all sectors including humans, food-producing animals, retail foods, and the environment, and also provision of the detailed methodology of the surveillance system. This includes combined analysis and reporting of these data across the two dimensions illustrated in Fig.3.1. The strategic framework for analyzing the impact of antimicrobial use on antimicrobial resistance both within sectors and across sectors is displayed in Fig.3.2. Scientific studies aimed at understanding the influence of antimicrobial use on antimicrobial resistance include microbiological and pharmacological studies, and population-based approaches such as epidemiology and ecology. Epidemiology is defined by Last as the “study of the distribution and determinants of health related states ... in specified populations” while ecology is defined as the “study of the relationships among living organisms and their environment (1).”

Fig.3.1 Schematic of full integration of surveillance of antimicrobial use and surveillance of antimicrobial resistance.

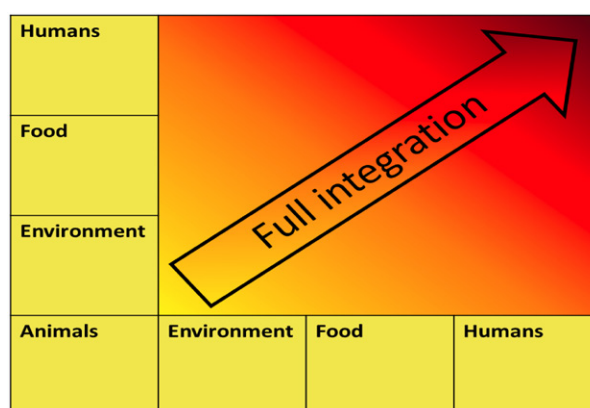
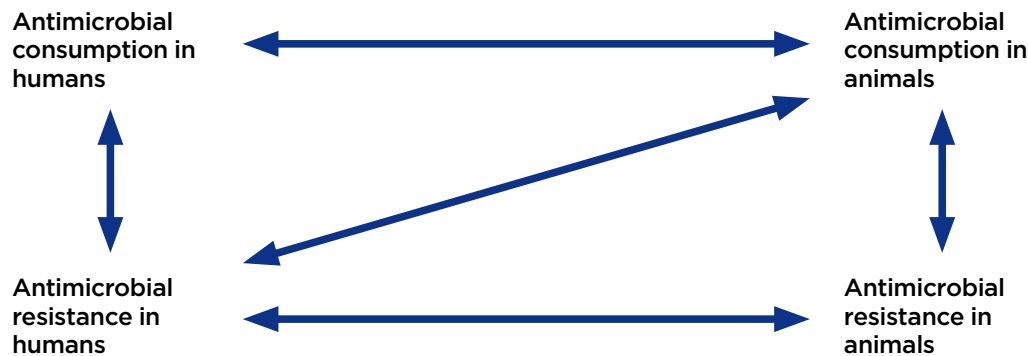


Fig.3.2 Example of strategic framework for analysing the impact of antimicrobial use on antimicrobial resistance across sectors.



Adapted from ECDC/EFSA/EMA First joint report on integrated analysis of the consumption of antimicrobial agents and occurrence of antimicrobial resistance in bacteria from humans and food-producing animals. 2015: Stockholm, Sweden. Available at: http://ecdc.europa.eu/en/publications/_layouts/forms/Publication_DispForm.aspx?List=4f55ad51-4aed-4d32-b960-af70113dbb90&ID=a1249.

The goal of a programme of integrated surveillance of antimicrobial resistance in foodborne bacteria should be timely integrated analysis and reporting of comparable surveillance data on antimicrobial use and antimicrobial resistance across all sectors to enable public health interventions which minimize the emergence and spread of antimicrobial resistance, and mitigate its impact. Ideally, the comparable surveillance data will be published in a single combined report summarizing antimicrobial use in animals and humans as well as antimicrobial resistance in animals, food, and humans. Such a report should include descriptions of integrated analysis and presentation of integrated tables and figures. There are multiple examples of approaches to achieve an annual report with combined analysis and reporting. In Canada, a single federal public health agency coordinates the Canadian Integrated Programme for Antimicrobial Resistance (CIPARS) and issues a single report annually. This remains the exception, rather than the rule, even among countries with more mature surveillance systems of antimicrobial resistance. Less integrated reports are still the norm in most countries. For example, in the United States of America, the National Antimicrobial Resistance Monitoring System (NARMS) is coordinated by three agencies affiliated with two federal departments. Each agency involved in NARMS issues its own annual report on antimicrobial resistance surveillance, with a single agency also issuing a summarized annual document on surveillance of antimicrobial use in food-producing animals. Such a document includes resistance endpoints in bacteria that are common to both humans and animals (e.g. commensal bacteria such as *Enterococcus* spp. and *E. coli* and pathogens such as *Campylobacter* spp. and nontyphoidal *Salmonella enterica*). In addition, it is desirable to include human-only and animal-only pathogens such as *Shigella* or *Salmonella Typhi* and *Mannheimia haemolytica* in humans and cattle, as respective examples.

A combined report of a programme of integrated surveillance of antimicrobial resistance among foodborne bacteria should include year-by-year estimates of the prevalence of resistance by bacterial species and sub-species, antimicrobials, and bacterial sources. Additionally, prevalence of resistance may be reported as MIC range, MIC 50, or MIC 90. Comparisons of these summary statistics by source and across years can also be graphically displayed. Other presentation options include the zone diameter, MIC measurement distributions and explorations of cross-resistance and multidrug resistance. Data on antimicrobial use, including population corrected data on antimicrobial use, can be presented in tabular form with year-by-year comparisons graphically displayed. Within-country versus between-country comparisons will be facilitated by using common statistical and derivable endpoints such as those generated by many European countries. Finally, box illustrations of integrated surveillance of antimicrobial

use and antimicrobial resistance in humans and animals, over time, can be useful for illustrating important resistance findings (2,3). Though such illustrations are often not necessarily routinely generated, these can prove especially useful in demonstrating relations between antimicrobial use and antimicrobial resistance at a more immediate scale. Additionally, these illustrations can be used to help communicate risk to stakeholders.

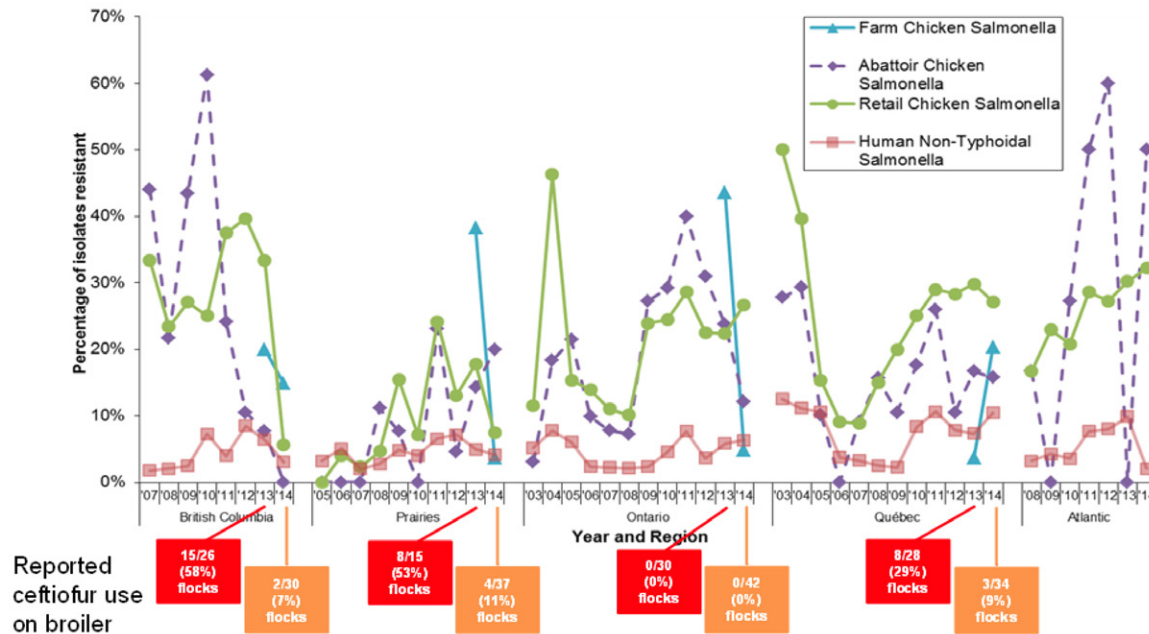
3.2. Examples of combined analysis and reporting of a programme of integrated surveillance of antimicrobial resistance in foodborne bacteria

Worldwide, there are few countries with combined analysis and reporting of a programme of integrated surveillance of antimicrobial resistance in foodborne bacteria that include surveillance of antimicrobial use and antimicrobial resistance. Common features of countries with combined analysis and reporting of a programme of integrated surveillance of antimicrobial resistance in foodborne bacteria include: 1) a longstanding surveillance of antimicrobial resistance that includes data from humans, animals, and food sectors; 2) surveillance of antimicrobial use which has reporting of antimicrobial use to a competent authority; and 3) regulatory policies intended to reduce or alter antimicrobial use patterns. Examples of countries with a combined analysis and reporting of a program of integrated surveillance of antimicrobial resistance in foodborne bacteria include Canada (CIPARS), Denmark (DANMAP), and the Netherlands (NethMAP/MARAN). The programmes in Canada, Denmark and the Netherlands have been utilized to monitor, analyze, and report on the occurrence of antimicrobial resistance in animals, food, and humans. These programmes have demonstrated the effects of interventions and changes in policies on antimicrobial use in animals and humans. At a regional level, there has been a European effort to fully integrate across multiple nations in the form of the Joint Interagency Antimicrobial Consumption and Resistance Analysis (JIACRA) Report. A report from this intensive large-scale project, combining the efforts and expertise of the European Centers for Disease Control, the European Food Safety Agency, and the European Medicines Agency, was published in January 2015.

Canada. The Canadian Integrated Programme for Antimicrobial Resistance Surveillance (CIPARS), established in 2002, is coordinated by the Public Health Agency of Canada, with contributions by other federal government departments, provincial and territorial ministries of health, agriculture, private industry, and academia. CIPARS generates annual reports, surveillance bulletins, industry reports, and ad hoc reports. There is currently no legislative requirement for data provision to CIPARS. Data for the majority of the surveillance components are provided voluntarily or, in the case of retail surveillance, CIPARS purchases the meat for culture and susceptibility testing. CIPARS has two general analysis meetings each year whereby analysts from each surveillance component describe the key findings (temporal trends and other notable findings). At these meetings, the analysts decide which key findings to conduct full analytic or descriptive integration of the data from all surveillance components. The integrated findings are presented at an annual national multisectoral stakeholder meeting, where stakeholders have the opportunity to provide additional relevant context to the results. The integrated findings are subsequently published as part of the CIPARS Annual Report (see Fig.3.3) and selected integrated findings are provided to the Canadian Antimicrobial Resistance Surveillance System reports. These reports include additional human antimicrobial use data and antimicrobial resistance findings for more human pathogens.



Fig.3.3 CIPARS – Regional trends in third generation cephalosporin use in chickens and third generation cephalosporin resistance in *Salmonella* from Canadian chicken(s) and people.



Adapted from ECDC/EFSA/EMA First joint report on integrated analysis of the consumption of antimicrobial agents and occurrence of antimicrobial resistance in bacteria from humans and food-producing animals. 2015: Stockholm, Sweden. Available at http://ecdc.europa.eu/en/publications/_layouts/forms/Publication_DispForm.aspx?List=4f55ad51-4aed-4d32-b960-af70113dbb90&ID=1249.

Denmark. The national programme of integrated surveillance of antimicrobial resistance in foodborne bacteria in Denmark (DANMAP) has published annual reports since 1996. The combined effort of the national public health institute (Serum Staten Institute), the National Food Institute and National Veterinary Institute of the Danish Technical University, and food-producing animal and agriculture and food sectors help to produce this annual report¹. This report provides summaries of antimicrobial use and antimicrobial resistance data across all sectors using standardized methods. Specific issues are highlighted each year through featured vignettes. Past vignettes have included ESBLs, livestock associated MRSA, a voluntary cessation of the use of 3rd and 4th generation cephalosporins by swine producers, as well as the Danish ‘yellow card’ system. Fig.3.4 shows several figures from DANMAP demonstrating the consequences of discontinuing, in 1996, antimicrobial growth promoter use in Denmark. Using the DANMAP data, Aarestrup *et al* also described the effects of this discontinuation (4). These DANMAP figures are among the best examples, worldwide, from annual reports of national surveillance systems, that demonstrate the relationship between antimicrobial use and antimicrobial resistance. The DANMAP reports are also unique concerning the ongoing efforts to separate out resistance data arising from imported foods versus foods derived from domestically reared food-producing animals. Such efforts are helpful in attempting to understand the relationship between antimicrobial use and antimicrobial resistance.

1 <http://danmap.org/Downloads/Reports.aspx>

Fig.3.4 DANMAP reporting of surveillance of antimicrobial use and surveillance of antimicrobial resistance, per year. Adapted from SSI/DTU, DANMAP 2010.

Fig. AP3.4.1 Resistance (%) to tetracycline among *Enterococcus faecium* and *Enterococcus faecalis* from pigs and the consumption of tetracyclines in pigs, Denmark

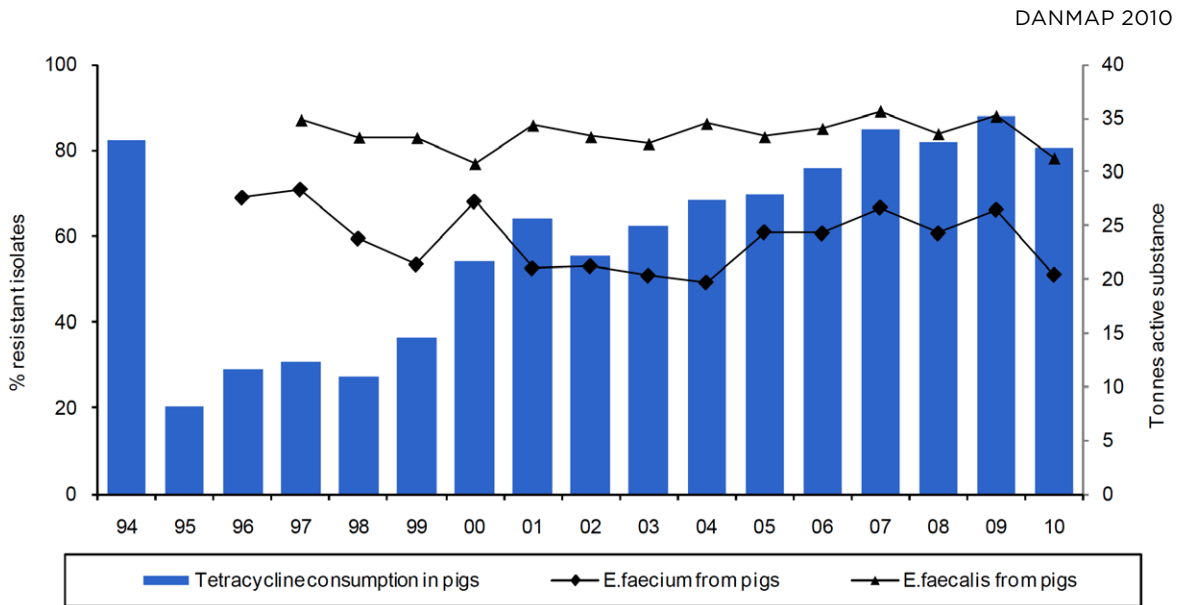


Fig.AP3.4.2 Resistance (%) to erythromycin among *Enterococcus faecium* and *Enterococcus faecalis* from pigs and the the consumption of macrolides in pigs, Denmark

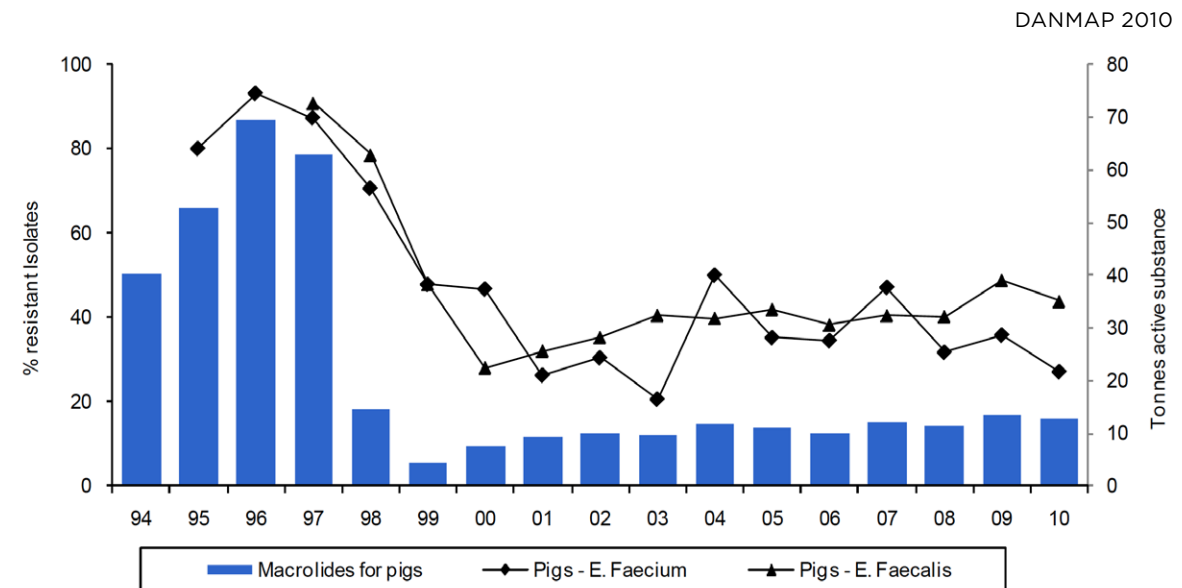


Fig.AP3.4.3 Resistance (%) to streptogramins in *Enterococcus faecium* from broilers and the consumption of virginiamycin, Denmark

DANMAP 2010

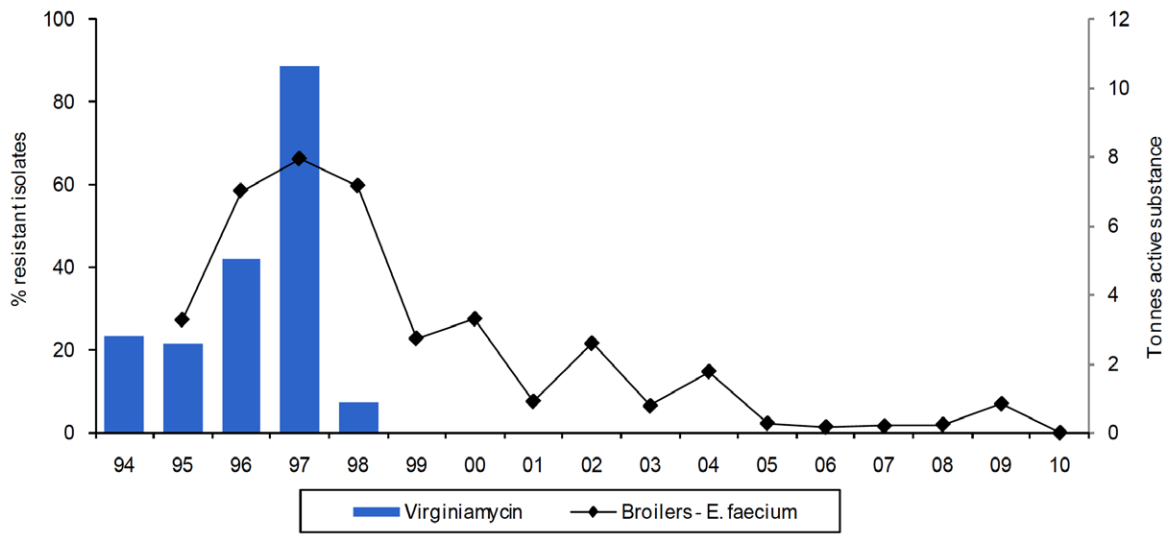


Fig.AP3.4.4 Resistance (%) to avoparcin in *Enterococcus faecium* and *Enterococcus faecalis* from broilers and the consumption of avoparcin, Denmark

DANMAP 2010

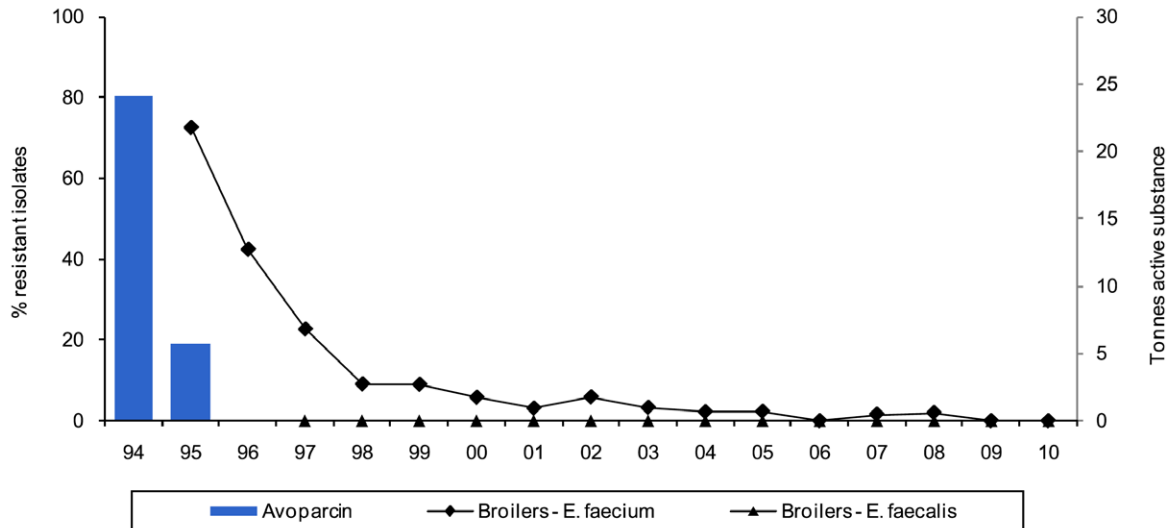


Fig.AP3.4.5 Resistance (%) to streptogramins in *Enterococcus faecium* from pigs and the consumption of virginiamycin, Denmark

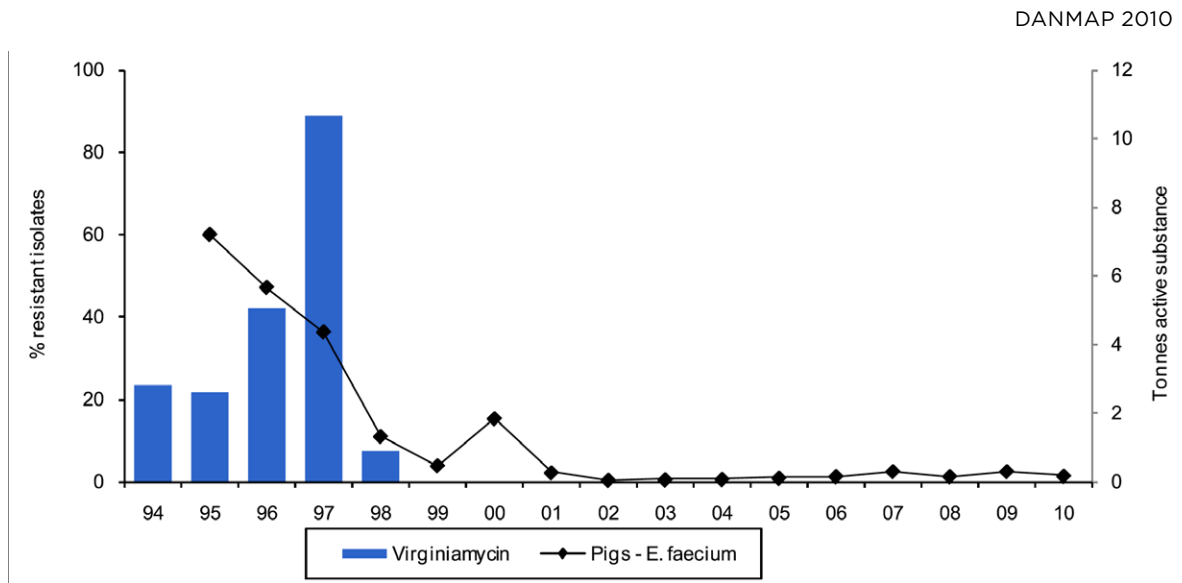
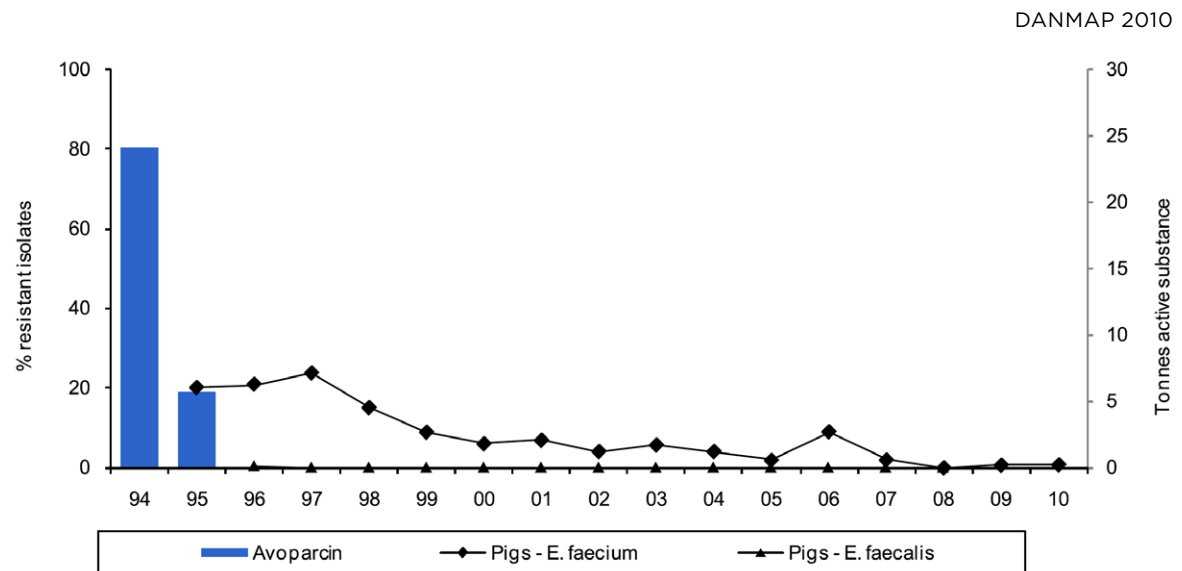


Fig.AP3.4.6 Resistance (%) to avoparcin in *Enterococcus faecium* and *Enterococcus faecalis* from pigs and the consumption of avoparcin, Denmark



Available at <http://www.danmap.org/downloads.reports.aspx>

The Netherlands. The annual report of the programme of integrated surveillance of antimicrobial resistance in foodborne bacteria in the Netherlands includes two components: NethMAP for surveillance among humans and MARAN for surveillance among animals and food sectors (5). In 2008, the Netherlands adopted policies and established goals for major reductions in antimicrobial use in food animal production. The MARAN annual report, which describes the progress toward achieving these goals, has increased in its importance and visibility. The MARAN report is issued by the Central Veterinary Institute with other partners, and is supplemented by the sDa (a Dutch veterinary medicines authority) report on veterinary and farm-level antimicrobial use data. sDa also issues standalone reports that include benchmarks for antimicrobial use by farmers and veterinarians. The MARAN report is issued by Wageningen Bioveterinary Research with other partners. The Netherlands Veterinary Medicines Authority (SDa) reports annually on antimicrobial sales and usage data on prescription level (national and farm level) (Fig.3.5, 3.6.). SDa also sets benchmarks for antimicrobial use in different animal (sub)sectors and on prescription level for veterinarians. MARAN reports have described the changes in antimicrobial resistance following the major decline in antimicrobial use resulting from the new policies put in place since 2008 (Fig. 3.7).

Fig.3.5 Annual sales of food animal antimicrobial classes in the Netherlands, 1999-2015.

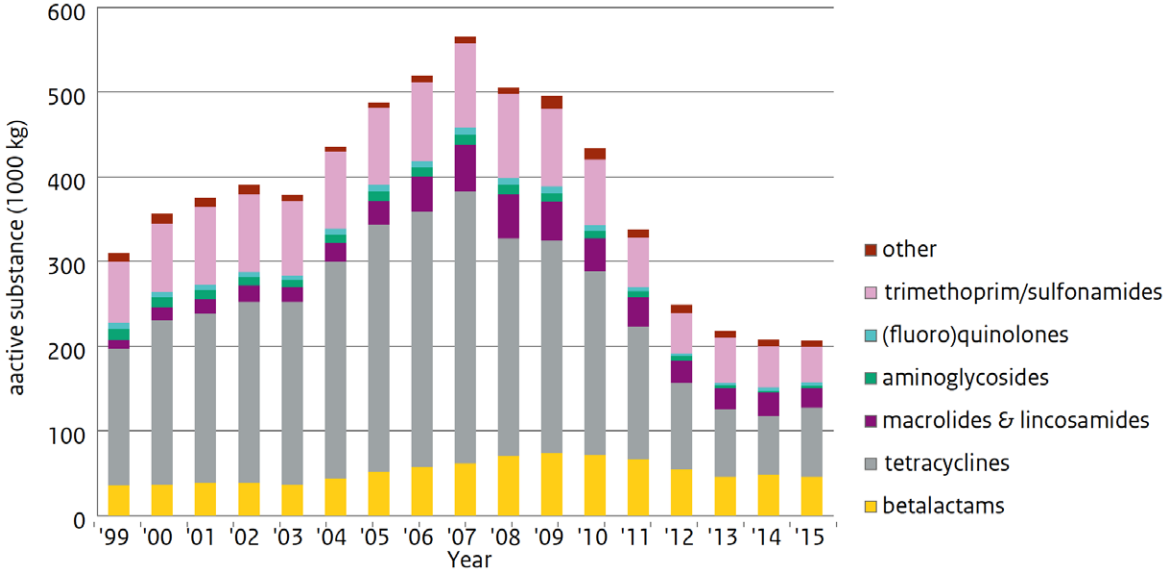


Fig.3.6 Defined Daily Doses for Animals, 2004-2015

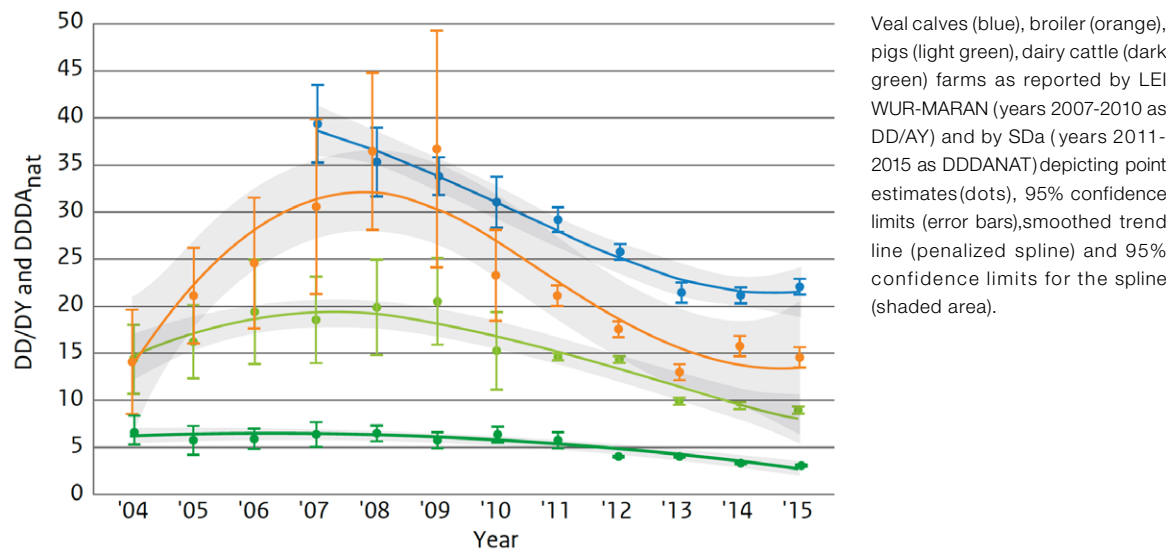
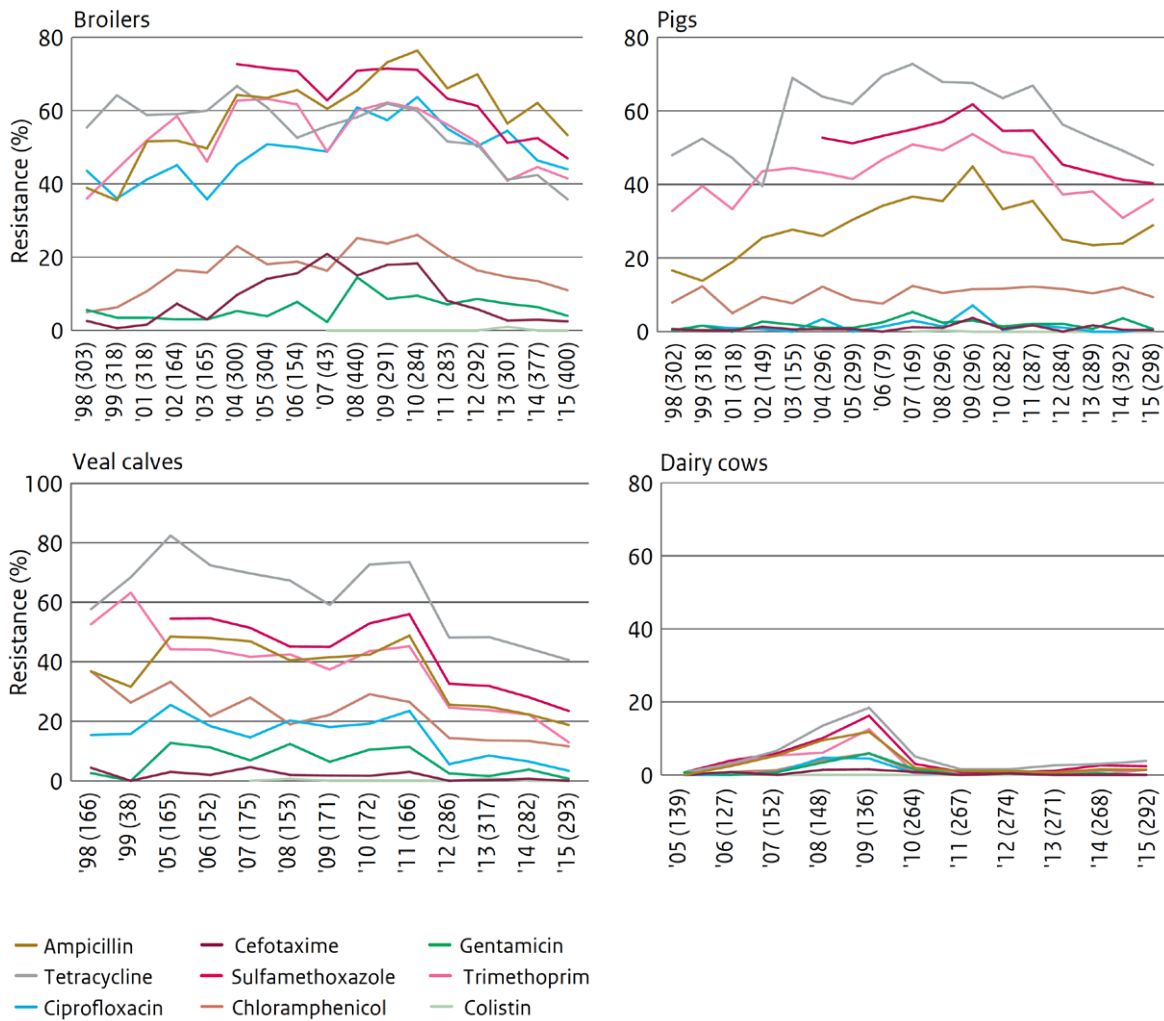


Fig.3.7 Trends for antimicrobial resistance among *Escherichia coli* in the Netherlands, by animal species, 1998-2015.



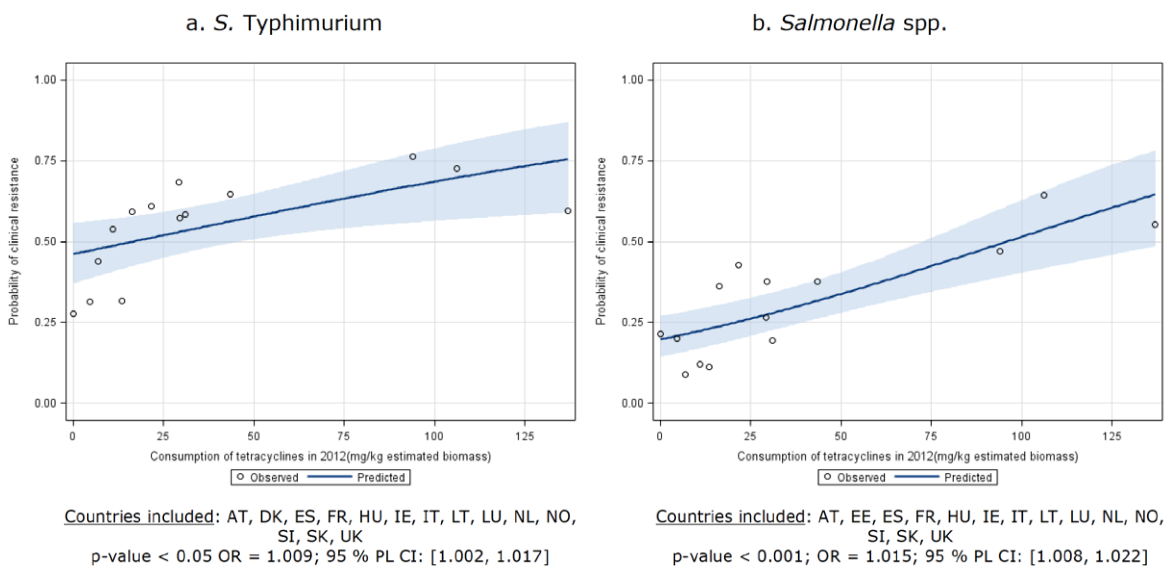
Figures 3.5, 3.6, 3.7 are available at:

http://www.rivm.nl/en/Documents_and_publications/Scientific/Reports/2016/juni/NethMap_

2016_Consumption_of_antimicrobial_agents_and_antimicrobial_resistance_among_medically_important_bacteria_in_the_Netherlands_MARAN_2016_Monitoring_of_antimicrobial_resistance_and_antibiotic_usage_in_animals_in_the_Netherlands_in_2015

European JIACRA Report. In January 2015, the first Joint Interagency Antimicrobial Consumption and Resistance Analysis (JIACRA) Report was released by three European public health agencies (6): the European Centers for Disease Prevention and Control (ECDC), the European Food Safety Authority (EFSA), and the European Medicines Agency (EMA). The JIACRA Report is an exhaustive effort that combines antimicrobial use and antimicrobial resistance data across animal, food and human sectors from 18 European countries. The report provides thorough documentation regarding data sources, data gaps, and methods employed. A major conclusion was that positive associations were observed between antimicrobial consumption in food-producing animals and occurrence of resistance in bacteria from such animals. The strongest associations between consumption and resistance in animals were related to antimicrobial use and effects on the indicator, *Escherichia coli*. When integrating the effects of antimicrobial use and antimicrobial resistance in animals (cephalosporins and fluoroquinolones) with antimicrobial resistance in humans, positive associations were found. Specifically, positive associations were found between the occurrence of resistance in the indicator, *E. coli*, originating from both animals and humans. However, no significant associations were observed between the consumption of 3rd and 4th generation cephalosporins in food-producing animals and the occurrence of resistance to 3rd and 4th generation cephalosporins in *Salmonella* from humans. Similarly, no significant associations were observed for consumption of fluoroquinolones in food-producing animals and the occurrence of fluoroquinolone-resistant *Campylobacter* spp. from humans. The JIACRA report does describe a positive association between consumption of macrolides in food-producing animals and the occurrence of macrolide resistance in *Campylobacter* spp. from humans. Additionally, the report describes a positive association between consumption of tetracyclines in food-producing animals and the occurrence of resistance to tetracycline in *Salmonella* spp. and *Campylobacter* spp. from humans (Fig.3.8).

Fig.3.8 JIACRA model of animal antimicrobial use and human resistance for *Salmonella* spp. and *Salmonella* serotype Typhimurium.



Available at: http://ecdc.europa.eu/en/publications/_layouts/forms/Publication_DispForm.aspx?List=4f55ad51-4aed-4d32-b960-af70113dbb90&ID=1249. Adapted from ECDC/EFSA/EMA First joint report on integrated analysis of the consumption of antimicrobial agents and occurrence of antimicrobial resistance in bacteria from humans and food-producing animals. 2015: Stockholm, Sweden

The JIACRA approach has several limitations. These limitations will need to be addressed when attempting to integrate the analysis and reporting of antimicrobial use and antimicrobial resistance surveillance data. As one example, the JIACRA report did not distinguish between antimicrobials used in food animal species, or between age groups, making population corrections difficult to achieve accurately. Data on antimicrobial use in humans are similarly problematic in the JIACRA report regarding antimicrobial use in hospitals or the community as well as hospital-acquired infections or community-acquired infections. Additionally, the JIACRA report relied on logistic regression to relate prevalence to human and animal resistance separately with use as the explanatory variables and a one year lag. It did not use prior year prevalence as a covariate. Such an approach could help to better understand if changes in antimicrobial resistance can be attributed to any observed change in antimicrobial use (as opposed to the continuation of previous patterns). Finally, the cumulative historical impacts of antimicrobial use are not accounted for in the JIACRA analysis. Emphasis, instead, is placed on concurrent use. Nevertheless, the JIACRA report provides an excellent example of integration of analysis and reporting of data from surveillance of antimicrobial use and surveillance of antimicrobial resistance from humans, animals and food.

3.3. Reporting options including risk communication

Reporting options employed in communicating the results of a programme of integrated surveillance of antimicrobial resistance in foodborne bacteria are diverse. In part, this reflects international differences in resource availability, national priorities including issues of concern within that country, and the maturity of the systems themselves. In many cases, country regulatory structures, jurisdictional matters, and resources dictate that separate reports be issued for each component of the programme (e.g. human, food and animal). While NARMS functions this way, composite executive summaries bring these reports together at a later date.

In other countries (e.g. CIPARS in Canada) a combined report is issued. Among the programmes with the most comprehensive analysis and reporting, such as in Denmark (DANMAP) and in the Netherlands (NethMAP and MARAN), each major public health or agri-food agency separate chapters and textboxes focusing on human, food and animal endpoints for both antimicrobial use and antimicrobial resistance, especially where non-zoonotic infectious agents are concerned.

There is no prescribed reporting requirement suggested herein. When data are sufficiently robust, or where a pressing concern dictates an analysis, such as presented in the 2015 JIACRA report, statistical approaches to inference as opposed to simple graphical and tabular descriptive approaches can be employed. Statistical approaches such as multivariable regression are less accessible to non-technical readers and require considerable attention to interpretation by lay persons and policy makers. Further, there is some risk in over-reliance on statistically derived results, especially if underlying limitations of the data and analysis are overlooked. Simple yet carefully crafted tabular and graphical displays are likely to remain the most highly effective forms of communication for the foreseeable future.

Risk communication arises naturally from issues pertaining to antimicrobial use and antimicrobial resistance, especially when there is a suggested negative impact on human health arising from antimicrobial use in animals. Considerations for effective risk communication, adapted and abridged from the first edition of this document (8) follow. Risk communication is broadly defined as the interactive exchange of information and opinions concerning hazards and risk management options among assessors, managers, consumers and other interested parties about threats to health, safety or the environment. The purpose of risk communication is to



increase knowledge about the nature and effects of risk, in order to promote collaborative work in the search for solutions. In the case of antimicrobial use and antimicrobial resistance, the latter is the identified hazard while the factors associated with antimicrobial use can be framed as quantifying its influence on the release, exposure, and even the consequence of the hazard to human or animal health. Risk communication can be divided into three general categories: core communication, consensus communication and crisis communication. Core communication is the sharing of information on health risks that have been identified through scientific research; generally, this is relatively non-controversial. Consensus communication aims to bring about consensus on how controversial risks should be managed. Finally, crisis communication focuses on communication in situations where sudden adverse events may pose a risk to public health.

A programme of integrated surveillance of antimicrobial resistance in foodborne bacteria will generate information of interest to multiple stakeholders, including: government risk managers, physicians, veterinarians, farmers, food manufacturers, retailers and consumers. For example, food producers may be concerned about the public disclosure of information regarding their production practices, including the use of antimicrobials in animal husbandry. On the other hand, consumers may be concerned that food is contaminated by resistant pathogens. Risk managers must be prepared to address the concerns of stakeholders at any point during the surveillance process. The implementation strategy is developed through an iterative process that feeds into the development of the risk communication plan, and will necessarily evolve over time (Table 3.1). Preliminary research and context analysis should identify the stakeholders and develop effective messages. A plan for implementation and leadership should be drawn up. Finally, continuous evaluation will allow for timely improvements to the strategy. The timing of communication is an important consideration as each stage of the programme will have different objectives including:

- Developing support for the programme and educating groups. In the early stages, a major task of risk communication is to encourage support for and participation in the programme, and to identify and educate groups of stakeholders.
- Ensuring smooth operation. Once the programme is running, it is especially important to keep open communications with the primary stakeholders, participants, and those involved in the day-to-day operations. This includes regularly asking about problems or concerns and responding to them in a timely manner.
- Keeping communication channels open. While data are being analysed, it is important to keep communications open, and to help stakeholders understand the process and the timeline for dissemination of results.
- Keeping all stakeholder groups informed about results. It is important that information is released to all stakeholder groups. Consideration should be given to the issues of concern expressed by each group. It is also important to recognize that each stakeholder will want details critical to them. Ideally, this should be done in a meeting, allowing all stakeholder groups to hear, question and respond to the information at the same time. This provides the best opportunity for stakeholder groups to assess the importance of information, as they can also hear other stakeholders' questions and comments. An open forum can also provide an opportunity for balanced media coverage, as different views are likely to be expressed.
- Continuously reviewing and evaluating communication materials and approaches. The effectiveness of the communications strategy should be regularly reviewed. Changes should be made to materials, spokespersons, or outreach methods as necessary. For integrated surveillance programmes, there are many stakeholders involved. As the surveillance programmes mature, the size of the annual report will likely increase. An increasingly large annual report can lead to difficulty sharing materials with stakeholders. Therefore, alternative forms of presentation and distribution of the results should be considered.
- Preparing for adverse events. The team should be prepared for any adverse events reported in the media so that they can respond rapidly.

Table 3.1. Risk Communications Plan

- Have a good understanding and description of the human health implications of AMR.
- Assess communications capacity and leadership, both among the project staff and externally.
- Identify stakeholders (including media, government departments, veterinarians, farmers, food processing industry, pharmaceutical industry, wholesale and retail food distributors and the general public) and establish the key concerns of each stakeholder group through dialogue.
- Together with the stakeholders, identify the target audiences for risk communication on antimicrobial resistance; establish participatory mechanisms to obtain input from the target audiences on their perception of the risks (including concerns, fears and worries), and tailor messages accordingly.
- Analyze the specific concerns to identify recurring themes and general concepts to be addressed.
- Develop key messages for each concern (both general and specific) of the stakeholders.
- For each message, identify key facts and information to support it.
- Test messages with the target audiences to whom they are directed.
- Plan for the broadcast of messages (including identifying suitable dissemination channels for the target audiences).

3.4. Example for starting a programme of integrated surveillance of antimicrobial resistance in foodborne bacteria

In order to align Member State approaches with the implementation principles of the Global Action Plan on antimicrobial resistance, a key initial task is to develop a smaller scale pilot project of surveillance of antimicrobial use and antimicrobial resistance (9). Such a pilot project can serve as a proof-of-principle that can assist each country to further refine and develop their own programme of integrated surveillance of antimicrobial resistance in foodborne bacteria. This programme can help address the public health concerns about resistance that originate from the use of antimicrobials in animals and humans. A first step in establishing a programme of integrated surveillance of antimicrobial resistance in foodborne bacteria is ensuring that the organizers are able to articulate the need for and the advantages of this programme. Useful considerations when building the case for the need for surveillance of antimicrobial resistance include understanding the human health burden of antimicrobial resistance and the international advantages with establishing such a programme (e.g. potential for enhancing trade).

The following can serve as suggested steps in developing a pilot project as a proof-of-principle for sustainable integrated surveillance programmes in countries with variable resource capacities.

3.4.1 Establishing governance

Establishing (or adapting) a multisectoral and technical working group assigned to the task of development, implementation, conduct, and follow-up of the pilot project. In many instances it



will be valuable to have an international advisory team to incorporate existing expertise in integrated surveillance systems as a first step. Such a group could be composed of experts from existing and established surveillance programmes and from international organizations such as WHO, OIE and FAO. In addition to this group, and following a stakeholder analysis mapping of the agri-food production, antimicrobial agent distribution systems, and public health systems, it can be very useful to initiate a stakeholder consortium comprised of public and private organizations to facilitate access to: 1) the sites of sampling (e.g. farm and slaughterhouses for animals, retail stores for meats, and hospitals or community care facilities for humans); 2) adequate laboratory capacities (e.g. both research and official laboratories from each of the public health, agri-food, and veterinary sectors); 3) relevant information on the food production system; and 4) identify and solicit potential sources of financial support. Official organizations likely to lead this process include the Ministries of Health, Food and Agriculture, Environment as well as each of their affiliated institutions.

3.4.2 Build a situation analysis

To determine strategic priorities, clearly stated objectives and triggered interventions are mandatory to fully assess antimicrobial use and antimicrobial resistance at a national level. The latter analysis facilitates establishment of each country's food chain risks and the capacity and gaps to address those risks in human and animal populations. The use of existing tools, such as those available from WHO, FAO, and OIE, can help to facilitate this step.

3.4.3 Planning

To determine the operational plan (activities, timetable, implementation arrangements, and responsible stakeholders) a benchmark of best practices developed by experienced organizations in Member States with well-developed and mature integrated surveillance systems (e.g. CIPARS, DANMAP, MARAN, NARMS) in terms of technical and technological approaches, infrastructure requirements, human resources, budgets, funding sources, international networks, and governance may be used. The collection of antimicrobial resistance and antimicrobial use data should follow suggestions in this document.

3.4.4 Plan activation

To overcome any lack of direct experience in large-scale inter-sectoral projects, a good practice is to engage the private sector (e.g. animal production industry and the pharmaceutical industry) early, with a strategy that includes the development of a proposal with added value from consumers, trading partners and more objective scientific endpoints. Economic benefits may include: the improvement of efficiency in animal and food production and increased herd health; improved data that is useful for in-depth investigations of specific health concerns; data on the economic impacts of the judicious use of antimicrobials; better knowledge of the epidemiology of pathogenic and indicator bacteria within the food system; and increased capacity for dissemination of the best international practices. A useful step towards the implementation of the pilot programme is the convening of a national workshop involving the constituted stakeholder consortium and International Advisory Team. This national workshop is where the value of the proposal, its likely benefits, expectations of various parties and obstacles to success, can be discussed.

3.4.5 Plan implementation

For the implementation of the pilot programme, a time frame is established on an annual basis. Ideally, every 12 months a complete evaluation with constructive feedback to stakeholders should be performed. The plan can be implemented in steps, with each success setting the

stage for implementation of subsequent components (e.g. start with antimicrobial resistance, then move to antimicrobial use; or, start with food, then animals, and then humans). As one example, the activities for the first year might be concentrated on farms and in appropriate abattoirs. The activities would include: recruitment of companies and farmers, contact and negotiation, discussion of planning and logistics of samplings, design of the antimicrobial resistance and antimicrobial use database, the collection of samples, laboratory testing and analysis, and reporting of antimicrobial resistance. For the second year, activities could include the creation of a census of retail stores, sourcing of human isolates from public health laboratories or health care facilities, sampling and laboratory analysis and testing and reporting of antimicrobial resistance. For the third year, the main tasks might include genotyping of isolates from human, food and animal origins. During these three years, capacity building is integrated with the specific outcome-oriented activities including writing reports for stakeholders and peer-reviewed articles.

3.4.6 Key success factors

The following factors are likely to be crucial for the successful early implementation of proof-of-concept activities: 1) ensuring confidentiality of protected and sensitive information from the general public while permitting public health and agricultural sectors to make use of such data in order to improve their own situation; 2) being transparent regarding each of the methods and processes of the surveillance programme, including effective communication of the results to all of the stakeholders. A highly accountable management programme contributes to achieving transparency; 3) building effective partnerships through active and ongoing negotiations among stakeholders in order to clarify goals and areas of responsibility, as well as promoting high levels of cooperation; and 4) obtaining scientific rigor by using widely recognized and validated sampling approaches and laboratory techniques employed by internationally recognized and established integrated surveillance programmes. Special emphasis should be put on building reliable databases and applying appropriate methodological analysis.

3.5. Evolution towards combined analysis and reporting of a programme of integrated surveillance of antimicrobial resistance in foodborne bacteria

Combined analysis and reporting of a programme of integrated surveillance of antimicrobial use and of antimicrobial resistance across all sectors (food-producing animals, retail foods, and humans) should be the goal for all countries. Progress towards such comprehensive analysis and reporting can be incremental; programmes can start separately, evolve, and develop towards integrated analysis and reporting. Combined analysis and reporting of integrated surveillance of antimicrobial use and of antimicrobial resistance can also be extended to a regional or multinational level, as described with the JIACRA report. This exhaustive analysis presented regional comparisons for food-producing animals and humans in Europe and attempted to relate antimicrobial use and antimicrobial resistance across sectors. It demonstrated how countries rank differently in antimicrobial use and in prevalence of antimicrobial resistance among different sectors.

Integrated approaches (e.g. where data on antimicrobial use and on antimicrobial resistance are compared), can begin at individual sites such as hospitals and farms instead of more nationally representative samples. Later, this can be combined into networks of similarly motivated sites. Countries can opt to begin at either end of aggregation (e.g. local versus national), depending on the infrastructure in place at the time of initiating a monitoring or



surveillance system. Likewise, countries can start with either surveillance of antimicrobial resistance or surveillance of antimicrobial use (or, both); however, the goal should be to ultimately achieve integration with combined reporting. For example, one entry level approach might start with point prevalence studies including antimicrobial use data from herds and hospitals that are aggregated from individual treatment records. National surveillance approaches could logically begin with established networks of public health laboratories for human isolates and animal slaughter facilities for animal isolates.

Some key points to consider:

- Expect to move from basic to sophisticated; that is, from standalone components to fully integrated analysis and reporting (e.g. start with human clinical isolates, progress to other resistance endpoints in food and animals, add sales data, and then consider on-farm monitoring of antimicrobial use);
- Aim toward national, then regional and finally international standards using the example of the JIACRA report as a regional effort;
- Consider the evolution of the integrated system (where to start, what to build towards, how to add components to achieve integrated surveillance and reporting on use and resistance in multiple sectors);
- Can have multiple starting points which evolve in parallel (e.g. start with import/ sales of antibiotics in certain regions, even while lacking resistance endpoints). Compare findings to those from other regions and inform what to expect when resistance endpoints are added in. Then build other sections into the programme.
- An excellent example of regional comparisons across multiple years using food, animal, human and use/resistance data is the JIACRA report released in January 2015.

3.6. References

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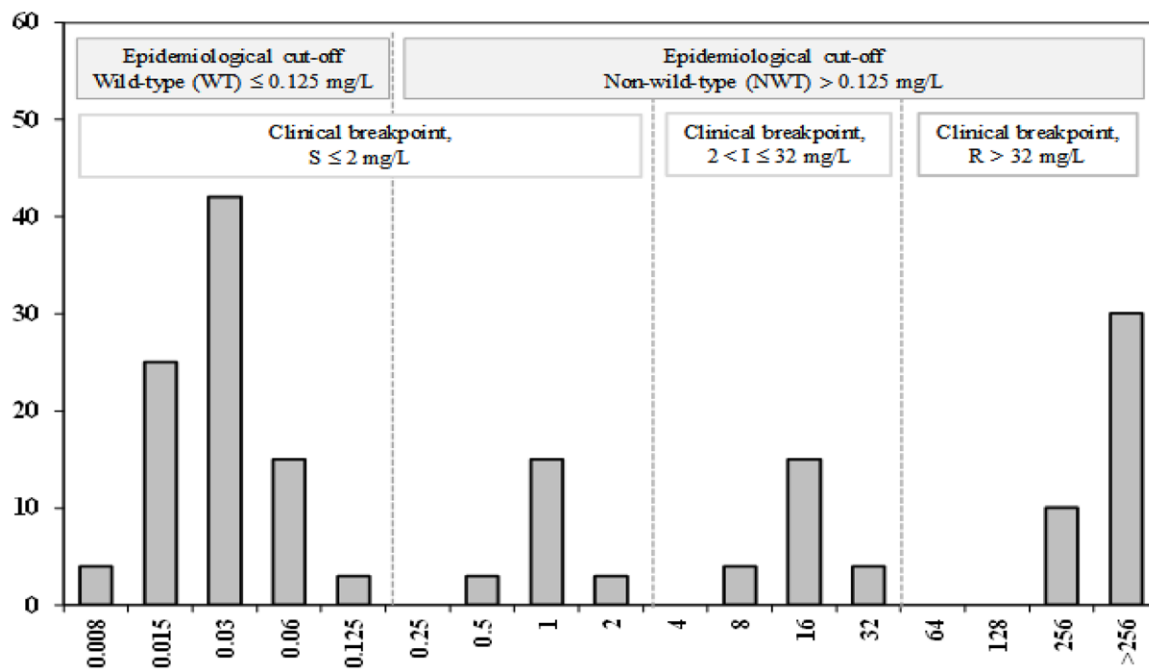


Appendix 1. Interpretation of antimicrobial susceptibility test results

Phenotypic determination of antimicrobial susceptibility of a bacterial isolate is important to ensure appropriate therapy for infections in animals and humans and to produce monitoring data on the occurrence of acquired resistance among bacteria in different reservoirs. Semi-quantitative methods for determining the MIC of an antimicrobial agent for a given bacterial isolate are currently the gold standard for antimicrobial susceptibility testing. A MIC is defined as the lowest drug concentration that visibly inhibits bacterial growth. In routine antimicrobial susceptibility testing, MICs are usually determined by serial two-fold drug dilutions, thus implying that the actual MIC of an isolate is only approximated. Indeed, the true MIC of an isolate growing at 1 mg/L, but not at 2 mg/L (thus, the recorded MIC is 2 mg/L), lies somewhere between 1 mg/L and 1.99 mg/L.

MIC values are interpreted using defined criteria to categorize bacterial isolates as susceptible or resistant, which is essential both for guiding appropriate clinical treatment and for comparing results from different monitoring programmes over time. However, interpretive criteria may differ among laboratories and countries, and also based on the purpose of the MIC determination. For example, MIC breakpoints appropriate for predicting clinical efficacy might differ from those used for surveillance purposes. An isolate might acquire reduced susceptibility to a given antimicrobial but still have a sufficiently low MIC to allow successful therapy. It is therefore important to differentiate between interpretative criteria used for clinical purposes (clinical breakpoints) and those used for monitoring (epidemiological cut-off values [ECOFFs]), as illustrated in the Figure A1.1 below.

Fig.A1.1 MIC distribution for a hypothetical organism-antimicrobial combination. S, susceptible; I, intermediate; R, resistant



According to clinical breakpoints, bacterial isolates are categorized as susceptible, intermediate or resistant to a given antimicrobial. Setting clinical breakpoints requires microbiological MIC data generated using standardized *in vitro* testing methods, pharmacokinetic and pharmacodynamic information generated from animal models and human studies, and outcome data from clinical efficacy trials. Clinical breakpoints provide guidance for antimicrobial treatment by implying that susceptible isolates are inhibited by the usually achievable concentrations of that antimicrobial agent when the recommended dosage is used for that site of infection. On the contrary, resistant isolates are not inhibited by the usually achievable concentrations of that antimicrobial with approved dosage schedules. The intermediate category provides flexibility for body sites where an antimicrobial is physiologically concentrated (thus likely implying clinical efficacy) or when higher-than-normal dosage of an antimicrobial can be used. In addition, it provides a buffer zone to account for day-to-day variability in *in vitro* antimicrobial susceptibility testing.

As defined by EUCAST, the ECOFF is the highest MIC for organisms devoid of phenotypically detectable acquired resistance mechanisms. Bacterial isolates are categorized as wild-type and non-wild-type. Wild-type isolates exhibit no resistance to a given antimicrobial whereas non-wild-type isolates have some type of acquired mechanism (e.g. mutations, acquisition of foreign DNA, up-regulation of an efflux pump, up-regulation of target production) reducing susceptibility to a given antimicrobial. Setting ECOFFs requires determination of the MIC distribution for organism-antimicrobial combinations using a large number of bacterial isolates of different geographical origin and collection times. Thus, the epidemiological cut-off takes into account exclusively microbiological properties independent of any consideration on drug dosages, site of infection, animal species and clinical efficacy.

Several national and international committees establish interpretive criteria for antimicrobial susceptibility testing. The most widely used are those provided by the Clinical and Laboratory Standards Institute (CLSI, www.clsi.org), which publishes methods for antimicrobial susceptibility testing and interpretive criteria based on clinical breakpoints. In Europe, the European Committee for Antimicrobial Susceptibility Testing (EUCAST, www.eucastr.org) provides both ECOFFs and clinical breakpoints which are freely available on the EUCAST website. It should be emphasized that interpretive criteria established by different organization may differ; therefore, it is important to specify the criteria used when reporting antimicrobial susceptibility test results. Continuous efforts to harmonize interpretive criteria for antimicrobial susceptibility test results should be pursued.

Being dependent exclusively on microbiological properties, ECOFFs provide a categorization of bacteria relative to antimicrobial susceptibility that is comparable across geographical areas, animal species and over time. Therefore, for monitoring purposes, the WHO Collaborating Centre for Antimicrobial Resistance in Foodborne Pathogens recommends and uses ECOFFs provided by EUCAST, as the reference standard for all organisms and antimicrobials.



Appendix 2. Quality control

The standardized and validated methods applied for the antimicrobial susceptibility testing, could be based on disk diffusion or MIC-methodologies (e.g. microbroth dilution, agar dilution). For both of these methods, internationally recognized consensus standards have been published by CLSI and ISO. The relevant official standard should always be consulted directly to ensure that all described steps are strictly followed for the method applied and not modified for local use. The following refers to some of the international standards that might be used but does not include a full list of the relevant standards. The listed items to include for observation and control should therefore not be regarded as a full checklist but as a summary of the parameters that are relevant for consideration when setting up or performing antimicrobial susceptibility testing.

The standards describe the preparation of the Mueller Hinton medium which should be prepared as described in the relevant standard for the selected antimicrobial susceptibility testing method. For the testing of *Campylobacter* (and other microorganisms), supplemented blood (defibrinated, lysed and quality assured horse blood or equivalent type of blood) is required for the Mueller Hinton medium. For the Mueller Hinton agar as well as Mueller Hinton broth, the acidity (pH) must be as described, between 7.2 and 7.4 (at room temperature). If necessary, cation-adjustment of the MH broth should be performed in accordance with the CLSI standards.

In addition to the type of agar, the depth of the agar is an important parameter to consider when performing antimicrobial susceptibility testing using the disk diffusion method including any antimicrobial gradient (epsilometer) test. The CLSI standard describes that the standard agar depth should be approximately 4 mm (M02-A12; Appendix B). Measuring the diffusion zones on too thin or too thick agar plates would render a diffusion zone larger or smaller, respectively, than those obtained using the standard criteria. Similarly, the quality of the disk used for disk diffusion should also be ensured (e.g. not exceeding the expiration date as this would affect the diffusion zones and result in an incorrect reading).

When preparing the inoculum, touch the top of 3-5 single colonies of the same morphological type from a non-selective agar plate culture. The level of inoculum on the agar plate or in the microbroth plate should be standardized using a densitometer or other means described in a relevant international standard to measure the density of the inoculum and compare it to a reference solution. For the purpose of a reference solution, the laboratory must apply a systematically replaced 0.5 McFarland standard, which should not be used after passing the expiration date. Additionally, when comparing the 0.5 McFarland standard to the test suspension, always perform the comparison with a McFarland standard in a tube of the same diameter and type as the test suspension. To check the inoculum preparation procedure and confirm the level of inoculum, periodically perform colony counts. The ISO method standard ISO 20776-1:2006 describes a method that should give a final cell number concentration of 5×10^5 CFU/mL (range 2×10^5 CFU/mL to 8×10^5 CFU/mL). Prepare a purity control that is incubated under the same conditions as the test isolates. For each of the microorganisms, the incubation time and temperature indicated in the relevant standard must be strictly followed. If the relevant standard for this parameter is not followed, the obtained results might be affected (e.g. by antimicrobial degradation) leading to a risk of obtaining deviating results.

Personnel trained to perform the reading of the incubated plates and/or microbroth panels must follow the reading procedures described for the method performed. Before reading the plate, verify that the purity control appears pure and if performing microbroth dilution, confirm growth in the positive control wells. If the growth is too weak, retesting might be necessary to obtain reliable results. The CLSI standards describe that the end-point interpretation should be

monitored periodically. The laboratory personnel who performs the tests should compare selected sets of tests independently read between observers. When performing disk diffusion, measurement readings from several individuals should not vary more than ± 2 mm (CLSI M02-A12). When performing broth dilution, all readers should agree within ± 1 two-fold concentration dilution (CLSI M07-A10). It is recommended that results be recorded on pre-prepared templates that request the following information: date, strain identity, incubation conditions, results of purity testing, staff member names reading the plates, and media batch. This template would facilitate data organization and allow for traceability.



Appendix 3. Interpretive criteria according to CLSI and EUCAST

(1) *Salmonella* and *E. coli*

High priority antimicrobial for testing	Species	Interpretative thresholds of AMR for MIC test (mg/L)			Range of concentrations (mg/L) No of wells in brackets	Interpretative thresholds of AMR for MIC test (mg/L)			Disk concentration CLSI/EUCAST
		CLSI Clinical break-point ^a	EUCAST Clinical break-point ^b	EUCAST ECOFF ^c		CLSI Clinical break-point ^a	EUCAST Clinical break-point ^b	EUCAST ECOFF ^c	
Amoxicillin	<i>Salmonella</i>	NA	> 8	> 4	ND	NA	NA	NA	NA
	<i>E. coli</i>	NA	> 8	> 8		NA	NA	NA	
Ampicillin	<i>Salmonella</i>	≥ 32	> 8	> 8	1-64 (7)	≤ 13	< 14	< 18	10 µg
	<i>E. coli</i>	≥ 32	> 8	> 8		≤ 13	< 14	< 14	
Azithromycin	<i>Salmonella</i>	≥ 32 ^e	NA	NA	2-64 (6)	≤ 12 ^e	NA	< 12	15 µg
	<i>E. coli</i>	NA	NA	NA		NA	NA	NA	
Cefepime	<i>Salmonella</i>	≥ 16	> 4	NA	0.06-32 (10)	≤ 18	< 21	NA	30 µg
	<i>E. coli</i>	≥ 16	> 4	> 0.125		≤ 18	< 21	< 28	
Cefotaxime	<i>Salmonella</i>	≥ 4	> 2	> 0.5	0.25-4 (5)	≤ 22	< 17	< 20	30 µg/5µg ⁱ
	<i>E. coli</i>	≥ 4	> 2	> 0.25		≤ 22	< 17	< 23	
Cefoxitin	<i>Salmonella</i>	≥ 32	NA	> 8	0.5-64 (8)	≤ 14	< 19	< 21	30 µg
	<i>E. coli</i>	≥ 32	NA	> 8		≤ 14	< 19	< 19	
Ceftazidime	<i>Salmonella</i>	≥ 16	> 4	> 2	0.5-8 (5)	≤ 17	< 19	< 20	30 µg/10 µg ⁱ
	<i>E. coli</i>	≥ 16	> 4	> 0.5		≤ 17	< 19	< 22	
Ceftriaxone	<i>Salmonella</i>	≥ 4	> 2	NA	ND	≤ 19	< 20	NA	30 µg
	<i>E. coli</i>	≥ 4	> 2	> 0.125		≤ 19	< 20	< 25	
Chloramphenicol	<i>Salmonella</i>	≥ 32	> 8	> 16	8-128 (5)	≤ 12	< 17	< 19	30 µg
	<i>E. coli</i>	≥ 32	> 8	> 16		≤ 12	< 17	< 17	
Ciprofloxacin	<i>Salmonella</i> ^k	≥ 4	> 0.06	> 0.064	0.015-8 (10)	≤ 15	NA	NA	5 µg
	<i>E. coli</i>	≥ 4	> 1	> 0.064		≤ 15	< 19	< 25	
Colistin	<i>Salmonella</i>	NA	> 2	> 2/ ⁿ 8 ⁿ	1-16 (5)	NA	NA	NA	NA
	<i>E. coli</i>	NA	> 2	> 2		NA	NA	NA	
Gentamicin	<i>Salmonella</i>	≥ 16	> 4	> 2	0.5-32 (7)	≤ 12	< 14	< 16	10 µg
	<i>E. coli</i>	≥ 16	> 4	> 2		≤ 12	< 14	< 16	
Imipenem	<i>Salmonella</i>	≥ 4	> 8	> 1	0.12-16 (8)	≤ 19	< 16	NA	10 µg
	<i>E. coli</i>	≥ 4	> 8	> 0.5		≤ 19	< 16	< 24	
Meropenem	<i>Salmonella</i>	≥ 4	> 8	> 0.125	0.03-16 (10)	≤ 19	< 16	< 27	10 µg
	<i>E. coli</i>	≥ 4	> 8	> 0.125		≤ 19	< 16	< 25	
Nalidixic acid	<i>Salmonella</i>	≥ 32	NA	> 16	4-128 (6)	≤ 13	NA	< 16	30 µg
	<i>E. coli</i>	≥ 32	NA	> 16		≤ 13	NA	< 19	
Sulfamethoxazole	<i>Salmonella</i>	NA	NA	> 256 ^d	8-1024 (8)	NA	NA	NA	NA
	<i>E. coli</i>	NA	NA	> 64		NA	NA	NA	
Tetracycline	<i>Salmonella</i>	≥ 16	NA	> 8	2-64 (6)	≤ 11	NA	< 17	30 µg
	<i>E. coli</i>	≥ 16	NA	> 8		≤ 11	NA	NA	
Trimethoprim	<i>Salmonella</i>	≥ 16	> 4 ^l	> 2	0.25-32 (8)	≤ 10	< 15 ^l	< 23	5 µg
	<i>E. coli</i>	≥ 16	> 4 ^l	> 2		≤ 10	< 15 ^l	< 20	
Trimethoprim-Sulfamethoxazole ^j	<i>Salmonella</i>	≥ 4	> 4	> 1	ND	≤ 10	< 13	NA	25 µg
	<i>E. coli</i>	≥ 4	> 4	> 1		≤ 10	< 13	< 16	

High priority antimicrobial for testing	Species	Interpretative thresholds of AMR for MIC test (mg/L)			Range of concentrations (mg/L) No of wells in brackets	Interpretative thresholds of AMR for MIC test (mg/L)			Disk concentration CLSI/EUCAST
		CLSI Clinical break-point ^a	EUCAST Clinical break-point ^b	EUCAST ECOFF ^c		CLSI Clinical break-point ^a	EUCAST Clinical break-point ^b	EUCAST ECOFF ^c	
Florfenicol	<i>Salmonella</i>	NA	NA	> 16	ND	NA	NA	NA	NA
	<i>E. coli</i>	NA	NA	> 16		NA	NA	NA	
Nitrofurantoin	<i>Salmonella</i>	≥ 128	NA	NA	ND	≤ 14	NA	NA	300 / 100 µg ⁱ
	<i>E. coli</i>	≥ 128	> 64	> 64		≤ 14	< 11	< 11	
Temocillin	<i>Salmonella</i>	NA	NA	NA	0.5-64 (8)	NA	NA	NA	30 µg
	<i>E. coli</i>	≥ 16/≥ 64 ^g	NA	NA		NA	NA	< 12 ^h	
Tigecyclinem	<i>Salmonella</i>	NA	> 2	> 1 ^f	0.25-8 (6)	NA	< 15	< 16	15 µg
	<i>E. coli</i>	NA	> 2	> 0.5		NA	< 15	< 18	

- a. CLSI Clinical breakpoint – Clinical and Laboratory Standards Institute resistance breakpoint.
 b. EUCAST Clinical breakpoint - European Committee on Antimicrobial Susceptibility Testing resistance breakpoint.
 c. EUCAST ECOFF – European Committee on Antimicrobial Susceptibility Testing epidemiological resistance cut-off value.
 d. No current EUCAST ECOFF is available for sulfamethoxazole, so the previous cutoff (>256) was maintained.
 e. Data from CLSI *Salmonella* Typhi only: Interpretive criteria are based on MIC distribution data.
 f. Data from EUCAST available for *Salmonella* Enteritidis, Typhimurium, Typhi and Paratyphi.
 g. Data from Woodford et al., J Antimicrob Chemother, doi:10.1093/jac/dkt383
 h. Data from Vanstone et al., J Antimicrob Chemother, doi:10.1093/jac/dkt248
 i. Note different concentration of disks, for interpretation by EUCAST and CLSI
 j. Trimethoprim- Sulfamethoxazole in the ratio 1:19. Breakpoints are expressed as the trimethoprim concentration.
 k. There is clinical evidence for ciprofloxacin to indicate a poor response in systemic infections caused by *Salmonella* spp. with low-level ciprofloxacin resistance (MIC >0.06 mg/L). The available data relate mainly to *Salmonella* Typhi but there are also case reports of poor response with other *Salmonella* species. Tests with a ciprofloxacin 5 µg disk will not reliably detect low-level resistance in *Salmonella* spp. To screen for ciprofloxacin resistance in *Salmonella* spp., use the pefloxacin 5 µg disk. Susceptibility of *Salmonella* spp. to ciprofloxacin can be inferred from pefloxacin disk diffusion susceptibility.
 l. Uncomplicated UTI only
 m. For Tigecycline broth microdilution MIC determination, the medium must be prepared fresh on the day of use
 n. The EUCAST ECOFF (>2) for colistin was applied for S. Typhimurium and other serotypes, except for S. Enteritidis and S. Dublin where ECOFF >8 was applied according to investigations presented in DANMAP 2011
 NA: Not Available
 ND: Not Determined

(2) *Campylobacter*

High priority antimicrobial for testing	Species	Interpretative thresholds of AMR for MIC test (mg/L)			Range of concentrations (mg/L) No of wells in brackets	Interpretative thresholds of AMR for Disk test (mm)			Disk concentration CLSI/ EUCAST
		CLSI Clinical breakpoint ^a	EUCAST Clinical breakpoint ^b	EUCAST ECOFF ^c		CLSI Clinical breakpoint ^a	EUCAST Clinical breakpoint ^b	EUCAST ECOFF ^c	
Ampicillin	<i>C. jejuni</i>	≥ 32 ^d	NA	> 8	ND	NA	NA	NA	NA
	<i>C. coli</i>	≥ 32 ^d	NA	> 8		NA	NA	NA	
Ciprofloxacin	<i>C. jejuni</i>	≥ 4	> 0.5	> 0.5	0.12-16 (8)	≤ 20	< 26	< 26	5 µg
	<i>C. coli</i>	≥ 4	> 0.5	> 0.5		≤ 20	< 26	< 26	
Erythromycin	<i>C. jejuni</i>	≥ 32	> 4	> 4	1-128 (8)	≤ 12	< 20	< 22	15 µg
	<i>C. coli</i>	≥ 32	> 8	> 8		≤ 12	< 24	< 24	
Gentamicin	<i>C. jejuni</i>	≥ 16 ^d	NA	> 2	0.12-16 (8)	NA	NA	NA	NA
	<i>C. coli</i>	≥ 16 ^d	NA	> 2		NA	NA	NA	
Tetracycline	<i>C. jejuni</i>	≥ 16	> 2	> 1	0.5-64 (8)	≤ 22	< 30	< 30	30 µg
	<i>C. coli</i>	≥ 16	> 2	> 2		≤ 22	< 30	< 30	

Lower priority antimicrobial for testing	Species	Interpretative thresholds of AMR for MIC test (mg/L)			Range of concentrations (mg/L) No of wells in brackets	Interpretative thresholds of AMR for Disk test (mm)			Disk concentration CLSI/ EUCAST
		CLSI Clinical breakpoint ^a	EUCAST Clinical breakpoint ^b	EUCAST ECOFF ^c		CLSI Clinical breakpoint ^a	EUCAST Clinical breakpoint ^b	EUCAST ECOFF ^c	
Clindamycin	<i>C. jejuni</i>	≥ 8 ^d	NA	> 0.5	ND	NA	NA	NA	NA
	<i>C. coli</i>	≥ 8 ^d	NA	> 1		NA	NA	NA	
Nalidixic acid	<i>C. jejuni</i>	≥ 32 ^d	NA	> 16	1-64 (7)	NA	NA	NA	NA
	<i>C. coli</i>	≥ 32 ^d	NA	> 16		NA	NA	NA	
Streptomycin	<i>C. jejuni</i>	NA	NA	> 4	0.25-16 (7)	NA	NA	NA	NA
	<i>C. coli</i>	NA	NA	> 4		NA	NA	NA	

- a. CLSI Clinical breakpoint – Clinical and Laboratory Standards Institute resistance breakpoint.
 b. EUCAST Clinical breakpoint - European Committee on Antimicrobial Susceptibility Testing resistance breakpoint.
 c. EUCAST ECOFF – European Committee on Antimicrobial Susceptibility Testing epidemiological resistance cut-off value.
 d. Breakpoints from Hakanen et al., J Antimicrob Chemother, doi:10.1093/jac/dkg489 and Lehtopulku et al., Antimicrob Agents Chemother, doi:10.1128/AAC.00898-09
 NA: Not Available
 ND: Not Determined

(3) *Enterococcus* spp.

High priority antimicrobial for testing	Species	Interpretative thresholds of AMR for MIC test (mg/L)			Range of concentrations (mg/L) No of wells in brackets	Interpretative thresholds of AMR for Disk test (mm)			Disk concentration CLSI/EUCAST
		CLSI Clinical break-point ^a	EUCAST Clinical break-point ^b	EUCAST ECOFF ^c		CLSI Clinical break-point ^a	EUCAST Clinical break-point ^b	EUCAST ECOFF ^c	
Ampicillin	<i>E. faecalis</i>	≥ 16	> 8	> 4	0.5-64 (8)	≤ 16	< 8	< 10	10 µg / 2 µg ^d
	<i>E. faecium</i>	≥ 16	> 8	> 4		≤ 16	< 8	< 10	
Vancomycin	<i>E. faecalis</i>	≥ 32	> 4	> 4	1-128 (8)	≤ 14	< 12 ^e	< 12	30 µg/5 µg ^d
	<i>E. faecium</i>	≥ 32	> 4	> 4		≤ 14	< 12 ^e	< 12	
Daptomycin	<i>E. faecalis</i>	≤ 4f	NA	> 4	0.25-32 (8)	NA	NA	NA	NA
	<i>E. faecium</i>	≤ 4f	NA	> 4		NA	NA	NA	
Erythromycin	<i>E. faecalis</i>	≥ 8	NA	> 4	1-128 (8)	≤ 13	NA	NA	15 µg
	<i>E. faecium</i>	≥ 8	NA	> 4		≤ 13	NA	NA	
Tetracycline	<i>E. faecalis</i>	≥ 16	NA	> 4	1-128 (8)	≤ 14	NA	NA	30 µg
	<i>E. faecium</i>	≥ 16	NA	> 4		≤ 14	NA	NA	
Ciprofloxacin	<i>E. faecalis</i>	≥ 4	> 4f	> 4	0.12-16 (8)	≤ 15	< 15 ^g	NA	5 µg
	<i>E. faecium</i>	≥ 4	> 4f	> 4		≤ 15	< 15 ^g	NA	
Chloramphenicol	<i>E. faecalis</i>	≥ 32	NA	> 32	4-128 (6)	≤ 12	NA	NA	30 µg
	<i>E. faecium</i>	≥ 32	NA	> 32		≤ 12	NA	NA	
Quinupristin/Dalfopristin	<i>E. faecalis</i>	≥ 4	NA	> 4h	0.5-64 (8)	≤ 15	NA	NA	15 µg
	<i>E. faecium</i>	≥ 4	> 4	NA		≤ 15	< 20	NA	
Linezolid	<i>E. faecalis</i>	≥ 8	> 4	> 4	0.5-64 (8)	≤ 20	< 19	< 19	30 µg/10 µg ^d
	<i>E. faecium</i>	≥ 8	> 4	> 4		≤ 20	< 19	< 19	
Gentamicin	<i>E. faecalis</i>	≥ 500	Note A	> 32	8-1024 (8)	6	Note A	< 8	120 µg/30 µg ^d
	<i>E. faecium</i>	≥ 500	Note A	> 32		6	Note A	< 8	
Tigecycline	<i>E. faecalis</i>	NA	> 0.5	> 0.25	0.03-4 (8)	NA	< 15	< 18	15 µg
	<i>E. faecium</i>	NA	> 0.5	> 0.25		NA	< 15	< 18	
Streptomycin	<i>E. faecium</i>	>1000	Note B	> 512	ND	6	Note B	NA	300 µg
	<i>E. faecalis</i>		Note B	> 128			Note B	NA	

Lower priority antimicrobial for testing	Species	Interpretative thresholds of AMR for MIC test (mg/L)			Range of concentrations (mg/L) No of wells in brackets	Interpretative thresholds of AMR for Disk test (mm)			Disk concentration CLSI/EUCAST
		CLSI Clinical break-point ^a	EUCAST Clinical break-point ^b	EUCAST ECOFF ^c		CLSI Clinical break-point ^a	EUCAST Clinical break-point ^b	EUCAST ECOFF ^c	
Teicoplanin	<i>E. faecalis</i>	≥ 32	> 2	> 2	0.5-64 (8)	≤ 10	< 16	< 16	30 µg
	<i>E. faecium</i>	≥ 32	> 2	> 2		≤ 10	< 16	< 16	
Nitrofurantoin	<i>E. faecalis</i>	≥ 128	> 64 ^g	> 32	ND	≤ 14	< 15	< 15	300µg/100µg ^d
	<i>E. faecium</i>	≥ 128	NA	> 256		≤ 14	NA	NA	

- a. CLSI Clinical breakpoint – Clinical and Laboratory Standards Institute resistance breakpoint.
 - b. EUCAST Clinical breakpoint - European Committee on Antimicrobial Susceptibility Testing resistance breakpoint.
 - c. EUCAST ECOFF – European Committee on Antimicrobial Susceptibility Testing epidemiological resistance cut-off value.
 - d. Note different concentrations of disk, for interpretation by EUCAST ECOFF and Clinical breakpoint by CLSI.
 - e. Vancomycin susceptible enterococci exhibit sharp zone edges and do not exhibit colonies in the inhibition zone. Examine zone edges with transmitted light (plate held up to light). If the zone edge is fuzzy, colonies grow within the zone or if you are uncertain, then perform confirmatory testing with PCR or report resistant even if the zone diameter is ≥ 12 mm. Isolates must not be reported susceptible before 24 h incubation.
 - f. Susceptibility breakpoint
 - g. Uncomplicated UTI only
 - h. The EUCAST ECOFF (>1) was not applied for quinupristin/dalfopristin (tradename synergid) according to investigations presented in DANMAP 2006
- NA: Not Available
ND: Not Determined

Note A: Gentamicin can be used to screen for high-level aminoglycoside resistance (HLAR). Negative test: Isolates with gentamicin MIC ≤ 128 mg/L or a zone diameter ≥ 8 mm. The isolate is wild type for gentamicin and low-level intrinsic resistant. For other aminoglycosides, this may not be the case. Synergy with penicillins or glycopeptides can be expected if the isolate is susceptible to the penicillin or glycopeptide. Positive test: Isolates with gentamicin MIC > 128 mg/L or a zone diameter < 8 mm. The isolate is high-level resistant to gentamicin and other aminoglycosides, except streptomycin which must be tested separately if required (see note 3/B). There will be no synergy with penicillins or glycopeptides.

Note B: Isolates with high-level gentamicin resistance may not be high-level resistant to streptomycin. Negative test: Isolates with streptomycin MIC ≤ 512 mg/L or a zone diameter ≥ 14 mm. The isolate is wild type for streptomycin and low-level intrinsic resistant. Synergy with penicillins or glycopeptides can be expected if the isolate is susceptible to the penicillin or glycopeptide. Positive test: Isolates with streptomycin MIC > 512 mg/L or a zone diameter < 14 mm. The isolate is high-level resistant to streptomycin. There will be no synergy with penicillins or glycopeptides.

(4) *Staphylococcus aureus*

High priority antimicrobial for testing	Species	Interpretative thresholds of AMR for MIC test (mg/L)			Range of concentrations (mg/L) No of wells in brackets	Interpretative thresholds of AMR for Disk test (mm)			Disk concentration CLSI/ EUCAST
		CLSI Clinical breakpoint ^a	EUCAST Clinical breakpoint ^b	EUCAST ECOFF ^c		CLSI Clinical breakpoint ^a	EUCAST Clinical breakpoint ^b	EUCAST ECOFF ^c	
Cefoxitin	<i>S. aureus</i>	NA	NA	> 4	ND	NA	< 22	< 22	30 μg
Chloramphenicol	<i>S. aureus</i>	≥ 32	> 8	> 16	2-64 (6)	≤ 12	< 18	< 18	30 μg
Ciprofloxacin	<i>S. aureus</i>	≥ 4	> 1	> 1	0.12-8 (7)	≤ 15	< 20	< 20	5 μg
Clindamycin	<i>S. aureus</i>	≥ 4	> 0.5	> 0.25	ND	≤ 14	< 19	< 22	2 μg
Erythromycin	<i>S. aureus</i>	≥ 8	> 2	> 1	0.25-16 (7)	≤ 13	< 18	< 21	15 μg
Gentamicin	<i>S. aureus</i>	≥ 16	> 1	> 2	0.25-16 (7)	≤ 12	< 18	< 18	10 μg
Linezolid	<i>S. aureus</i>	≥ 8	> 4	> 4	ND	≤ 20	< 19	< 19	30 $\mu\text{g}/10 \mu\text{g}^{\text{d}}$
Oxacillin	<i>S. aureus</i>	≥ 4	NA	> 2	ND	NA	NA	NA	NA
Quinupristin/Dalfopristin	<i>S. aureus</i>	≥ 0.25	NA	NA	0.06-16 (9)	≤ 28	NA	NA	10 units
Dalfopristin	<i>S. aureus</i>	≥ 4	> 2	> 1	ND	≤ 15	< 18	< 21	15 μg
Rifampicin	<i>S. aureus</i>	≥ 4	> 0.5	> 0.032	ND	≤ 16	< 23	< 25	5 μg
Sulfamethoxazole	<i>S. aureus</i>	$\geq 512^{\text{d}}$	NA	> 128	32-512 (5)	$\leq 12^{\text{d}}$	NA	NA	300 μg^{e}
Tetracycline	<i>S. aureus</i>	≥ 16	> 2	> 1	0.5-32 (7)	≤ 14	< 19	< 22	30 μg
Trimethoprim	<i>S. aureus</i>	≥ 16	$> 4^{\text{f}}$	> 2	0.5-32 (7)	≤ 10	$< 14^{\text{f}}$	< 19	5 μg
Vancomycin	<i>S. aureus</i>	≥ 16	> 2	> 2	ND	NA	NA	NA	NA

- a. CLSI Clinical breakpoint – Clinical and Laboratory Standards Institute resistance breakpoint.
- b. EUCAST Clinical breakpoint - European Committee on Antimicrobial Susceptibility Testing resistance breakpoint.
- c. EUCAST ECOFF – European Committee on Antimicrobial Susceptibility Testing epidemiological resistance cut-off value.
- d. Note different concentrations of disk, for interpretation by EUCAST ECOFF and Clinical breakpoint by CLSI. Organisms with resistant results towards Linezolid from disk diffusion should be confirmed using MIC.
- e. Data refer to the class, sulfonamides.
- f. Uncomplicated UTI only NA: Not Available ND: Not Determined



Appendix 4: The use of whole genome sequencing in antimicrobial resistance surveillance

Whole Genome Sequencing (WGS) of bacteria has become a fast and increasingly more affordable technology that is rapidly being adopted by surveillance and diagnostic laboratories around the world. Along with the drop in reagent and instrument costs, bioinformatics tools are quickly evolving to simplify the analytical processes, making it possible to determine and evaluate the entire DNA sequence of a bacterium in a short time. In integrated antimicrobial resistance surveillance, the detailed genetic data provided by WGS technology greatly enhance the ability of public health investigators to identify outbreaks, trace pathogens back to their source, and understand, in detail, the spread of antibiotic resistance from agriculture use environments through the food supply. It is anticipated that WGS will replace several analytic methods currently used to track and treat infectious diseases. These include pulsed-field gel electrophoresis (PFGE), serotyping, antibiotic resistance screening, plasmid typing, virulence profiling, and various targeted PCR processes, among others. Each of these traditional methods requires specialized training, dedicated instrumentation and the need to maintain a variety of costly reagents. The possibility of WGS to supplant all these methods will greatly enhance integrated antimicrobial resistance surveillance. It will speed analysis and reporting, and by reducing costs, enable expanded testing to increase the number and sources under surveillance.

Given the demonstrated power of WGS, programmes of integrated surveillance of antimicrobial resistance in foodborne bacteria have already begun to use it to conduct surveillance of antimicrobial resistance, enhance real-time analysis of outbreak investigations, and conduct research on highly resistant or emergent patterns or unusual strains. Studies comparing the results on in vitro antimicrobial susceptibility testing and WGS showed that the presence of known resistance determinant is highly (99%) correlated with clinical resistance. The ability to rapidly sequence different bacterial species isolated from different sources (humans, animals, foods and the environment) in the integrated surveillance will allow us to better understand how selection pressure plays a role in resistance development, dissemination and persistence in different environments. This is illustrated in a study examining increasing gentamicin resistance in *Campylobacter* from the United States of America. In this example, a sharp increase in resistance was caused by seven different alleles, several not previously detected in the genus and that would have been missed by traditional PCR methods. The resolution to distinguish bacteria with identical resistance patterns caused by different mechanisms is a new level of detail not easily acquired before that will refine our understanding of the drivers of resistance.

One of the most powerful advantages of WGS is that it eliminates the need to return to the laboratory to conduct retrospective analyses in response to emerging hazards. This was illustrated in November 2015 by the discovery of colistin resistance in swine *E.coli* from China. The gene conferring this resistance (*mcr-1*) was present on a mobile plasmid. This was the first identified instance of transmissible resistance to this important drug. This finding got the attention of the world. Following this, institutes in numerous countries began to look for the *mcr-1* gene in their strain collections. For those with large WGS databases, there was no need to regrow banked cultures and conduct the series of laboratory processes to detect *mcr-1*. It was a bioinformatics exercise to examine the WGS databases, which can be done in just a few hours. By providing definitive genotype information, WGS offers the highest practical resolution for detecting and characterizing the full complement of resistance determinants, whether acquired exogenously or arising by mutation, including resistance to antibiotics not routinely tested. In doing so, WGS makes it possible to quickly evaluate the nature and magnitude of emerging resistance hazards, and can serve a vital function to integrated surveillance of antimicrobial resistance.

