Doctoral thesis

Description of surveillance and monitoring systems on AMU and AMR in European countries and comparison of antimicrobial use and resistance data on clinical and non-clinical *E. coli* isolates from livestock in countries

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Description of surveillance and monitoring systems on AMU and AMR in European countries and comparison of antimicrobial use and resistance data on clinical and non-clinical *E. coli* isolates from livestock in countries

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“He knows not his own strength

that has not met adversity”

(Ben Jonson)

To my daughters,

The light of my days

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Abbreviation list

Abbreviation	Term
AMU	Antimicrobial use
AMUsage	Antimicrobial usage
AMR	Antimicrobial resistance
ARDIG	Antimicrobial Resistance Dynamics
AST	Antimicrobial Susceptibility Testing
CBP	Clinical Break-Point
CLSI	The Clinical & Laboratory Standards Institute
COIPARS	Colombian Integrated Surveillance Program for Antimicrobial Resistance
DANMAP	The Danish Integrated Antimicrobial Resistance Monitoring and Research Programme
DDD	Defined Daily Dose
ECDC	European Centre for Disease Prevention and Control
ECOFF	Epidemiological Cut OFF
EFSA	European Food Safety Authority
EMA	European Medicines Agency
ESAC-Net	European Surveillance of Antimicrobial Consumption Network
ESVAC	European Surveillance of Veterinary Antimicrobial Consumption
EU	Europe/European
EUCAST	The European Committee on Antimicrobial Susceptibility Testing
GAP	Global Action Plan
HGT	Horizontal Gene Transfer
IZD	Inhibition Zone Diameter
JIACRA	Analysis of antimicrobial consumption and resistance
MIC	Minimum Inhibition Concentration
NAP	National Action Plan
NARMS	National Antimicrobial Resistance Monitoring System in the United States
NRI	The Normalized Resistance Interpretation method
SGSS	Second Generation Surveillance System
SOP	Standard Operating Procedure
STAR-PU	Specific Therapeutic Group Age-sex weightings Related Prescribing Units
TF	Therapy Frequency
WHO	World Health Organization

WP1	Work package 1
SIR	Susceptible-Intermediate-Resistant

Chapter 1: Introduction

The aims of this thesis are to:

- Describe surveillance and monitoring systems on antimicrobial use (AMU) and antimicrobial resistance (AMR) in European countries.
- Describe and compare resistance data of clinical and non-clinical *E. coli* isolates from livestock in countries.
- Describe the association of AMU and year with AMR.

The main hypothesis challenged in chapter 4 and 5 is that the resistance level of *E. coli* from animals is higher in clinical isolates than in non-clinical isolates as diseased animals might carry resistant bacteria to regular antimicrobials. In chapter 4, it was feasible to include the AMU as an explanatory variable and, therefore, a second hypothesis was that there is an association between AMU and AMR. The analysis of the differences between clinical and non-clinical isolates would ease the interpretation of those analyses that compare different populations by using different isolate types such as the JIACRA reports (EFSA/EMA/ECDC 2021, EFSA/EMA/ECDC 2017, EFSA/EMA/ECDC 2015).

The final goal of this work is to provide recommendations for improved “One Health” surveillance at the European level.

Chapter 2 provides a general overview of AMR highlighting the value and usefulness of this work. Chapter 3 contains a review of monitoring antimicrobial resistance and drug usage in the human and livestock sector and foodborne antimicrobial resistance in six European countries. In chapter 4, phenotypical antimicrobial resistance data of clinical and non-clinical *E. coli* from German poultry were analysed statistically between 2014 and 2017. Additionally, AMR changes over time and the association of changes in AMU with changes in AMR were also included in the analyses. In Chapter 5, comparisons of phenotypical antimicrobial resistance were performed in clinical and non-clinical *E. coli* isolates from broilers, turkeys and calves in four European countries. Chapter 6 provides a general discussion of this work.

This work originated from the work package 1 (WP1) of the Antimicrobial Resistance Dynamics (ARDIG) project, a One Health European Joint Programme. ARDIG WP1 collected available consumption data from humans and livestock together with phenotypical resistance data of *E. coli* from urinary samples in humans, livestock and meat from Germany, Spain, France, the Netherlands, Norway and the United Kingdom from 2014 to 2017.

Chapter 2: Literature

Antimicrobials are remedies used to combat infections. These drugs are highly relevant for the economy and the health status saving animal and human lives, increasing life span expectation and facilitating medical advances. The antimicrobial era began when Alexander Fleming discovered penicillin in 1928 (Fleming 1929), however, infection treatments were previously well documented in many countries such as Egypt, Greece and China (Sengupta et al. 2013).

Different types of antimicrobial treatments are historically used in humans and animals. Therapeutic and prophylactic treatments are applied in the human sector while a wider variety is shown in the animal sector including therapeutic, prophylactic, metaphylaxic and growth promotion treatments. Therapeutic treatments use high doses of prescribed antimicrobials to the diseased individual/population while prophylaxis treatments consist of the administration of antimicrobials to the healthy individual/population in order to prevent bacterial infections. In the animal sector, the metaphylaxis strategy consists of treating clinically healthy animals suspected of being infected with an organism. Growth promotion is based on the administration of non-therapeutic doses of antimicrobials to livestock. These sub-therapeutic doses enhance animal growth although the action mechanism has not been fully clarified (Morel 2019). Due to the evidence that sub-therapeutic antimicrobial doses favour the AMR appearance (Li et al. 2017), use of antimicrobials for growth promotion has been banned in several regions such as Europe.

Resistance to antimicrobials is a natural biological event that causes the microorganisms to lose sensitivity to the effect of the antimicrobial that was previously effective in treating it. Those bacteria with resistance genes that protect them from different antimicrobial classes are called multi-resistant bacteria. Resistance to antimicrobials has frequently been reported in bacteria from permafrost soils where it is preserved for hundreds of years evidencing that AMR is ancient (Perry et al. 2016, D'Costa et al. 2011). However, AMR has become a worldwide issue as the frequency and diversity of resistance genes have massively increased mainly due to the use of drugs in the last decades (World Health Organization (WHO) 2014).

Some bacteria are naturally resistant to specific types of antimicrobials. This natural resistance can be classified as intrinsic or induced. (i) Intrinsic resistance is always expressed in the species and is not related to previous antimicrobial exposure (Martinez 2014). The most common intrinsic resistance mechanisms are: (a) The reduced permeability of the outer membrane and (b) the natural activity of efflux pumps (Cox and Wright 2013). (ii) Induced

resistance is shown when bacteria carry the resistance genes naturally but they are only expressed after antimicrobial exposure. The efflux pump is a common mechanism of induced resistance (Cox and Wright 2013, Fajardo et al. 2008).

Bacteria can also acquire resistance by a genetic mutation or by acquiring resistance from other bacteria. In bacteria, the average mutation rate is 1 per 10^6 to 10^9 cell divisions. Most of them are deleterious to the cell (Davies and Davies 2010). Some of these mutations promote resistance to antimicrobials and can be transmitted to descendant cells. In most cases, non-deleterious gene mutations to the cell that increase resistance of bacteria entail fitness cost (Melnyk et al. 2015). However, those mutations with little or no fitness cost are more likely to persist in the environment in the antimicrobial absence (Melnyk et al. 2015).

Acquisition of resistance genes can also be caused by homologous recombination (i.e. Horizontal Gene Transfer (HGT) that can occur by (1) transformation, (2) transduction or (3) conjugation) or by non-homologous recombination (i.e. transposition)). By homologous recombination, bacteria acquire a DNA fragment similar to a part of the genome. In the case of transposition, a non-homologous recombination, the fragment acquired by bacteria (i.e. integrins and transposons) differs to the structure of the genetic material of the bacteria.
The most common route for transmission of resistance genes is conjugation (i.e. plasmids) while transduction (i.e. bacteriophages) is rare (Reygaert 2018).

Surveillance and monitoring systems

Interest in AMR has been changing over time. Likewise, the motivations that have given rise to this interest have also varied. As an example, a study identified in the United States five main periods where the interest in AMR was changing (Podolsky 2018). In recent years, AMR is emerging rapidly jeopardizing the drug´s usefulness (Buckner et al. 2018). Death proportions from AMR could surpass the cancer mortality incidence by the year 2030 (Aminov 2017). It is estimated that AMR will cause only in the European Economic Area/Europe region 1.3 million deaths between 2015 and 2050 (Driss Ait Ouakrim et al. 2018).

Worldwide strategies such as the Global Action Plan (GAP) of the World Health Organization (WHO) (World Health Organization (WHO) 2015a), the EU Action on Antimicrobial Resistance (European Commission 2016) and National Action Plans (NAP) (World Health Organization (WHO) 2015b) have been implemented to limit the spread and development of AMR. Surveillance and monitoring systems are key elements to assess and control the global trends of AMU and AMR. Zoonotic and indicator bacteria are of special interest. These systems are part of national and global strategies collecting reliable and quality data to: (a) document the situation; (b) identify trends; (c) set up the basis for risk assessment and interventions; (d)

assess effects of efforts carried out; (e) associate AMU and AMR; (f) focus and target the research (FAO/OIE/WHO 2003); and (g) advise on veterinary treatments and antimicrobial stewardship (Sanders et al. 2020). However, not all countries have these kind of systems in place. Therefore, it is not feasible to assess the data across countries worldwide.

Guidelines and standards have already been developed by the World Health Organization (WHO), the World Organisation for Animal Health (OIE) and the FAO/WHO Codex Alimentarius to support the national implementation of AMU and AMR systems in humans, animals and food systems (World Health Organization (WHO) 2021b, OIE 2019, World Health Organization (WHO) 2017a, Food and Agriculture Organization of the United Nations (FAO) 2011). However, they do not necessarily consider cross sectoral issues (Interagency Coordination Group on Antimicrobial Resistance (IACG) 2018). No guidelines are available, so far, for monitoring AMR and AMU in plants, in the environment and their relationship to food production (Interagency Coordination Group on Antimicrobial Resistance (IACG) 2018). There are some initiatives addressing AMR in several sectors at national and international level such as: (a) the Joint Interagency Antimicrobial Consumption and Resistance Analyses (JIACRA) of data on humans and livestock in Europe (EFSA/EMA/ECDC 2021, EFSA/EMA/ECDC 2017, EFSA/EMA/ECDC 2015). (b) The National Antimicrobial Resistance Monitoring System (NARMS) in the United States, that includes data from surveillance in humans, animals and food (U.S. Food $ Drug Administration 2021). (c) The Danish Integrated Antimicrobial Resistance Monitoring and Research Programme (DANMAP) (Technical University of Denmark (DTU) 2019) and (d) the Colombian Integrated Surveillance Program for Antimicrobial Resistance (COIPARS) (Donado-Godoy et al. 2015).

Data on AMR are collected from diseased and healthy populations in Europe. Human data on AMR are mostly collected from diseased individuals. On the European level, this data collection is performed by the European Centre for Disease Prevention and Control (ECDC). Non-clinical data are also collected but only for specific resistant bacteria such as Methicillin Resistant *Staphylococcus aureus* (MRSA) (Kinross et al. 2017, Kock et al. 2014).

On the contrary, livestock data on AMR in Europe are mainly collected on non-clinical isolates. Data collection on non-clinical isolates is performed in Europe by the European Food Safety Authority (EFSA) (European Food Safety Authority (EFSA) 2020). This collection of data on zoonotic and indicator bacteria is harmonised due to Decision 2020/1729/EU. Additionally, the private sector funds the VetPath and MycoPath initiatives that collect limited data on clinical isolates from diagnostic submissions from livestock in Europe (Schrijver et al. 2018, El Garch et al. 2016). Some countries also collect resistance data on clinical isolates at national level such as Germany, the United Kingdom and France.

Escherichia coli are Gram-negative commensals in the intestinal tract of humans and animals. They can also be pathogens. Antimicrobial resistance carried by *E. coli* can be spread horizontally to other bacteria (Djordjevic et al. 2013). *Escherichia coli* are widely accepted as AMR indicator (European food Safety Authority (EFSA) 2019).

Resistance differences have been shown between clinical and non-clinical *E. coli* isolates at the descriptive level (Aasmäe et al. 2019). This study showed a higher resistance level of clinical isolates compared to non-clinical isolates of dairy cows and swine. However, no statistical studies were performed in livestock between both isolate types until 2020. The analysis of the differences between clinical and non-clinical isolates would ease the interpretation of those analyses that compare different populations by using different isolate types such as the JIACRA reports (EFSA/EMA/ECDC 2021, EFSA/EMA/ECDC 2017, EFSA/EMA/ECDC 2015).

Antimicrobial resistance and microorganism virulence are showing frequently a positive or negative association (Cepas and Soto 2020). This relationship may benefit the microorganism conferring features that favour the survival in different niches to different selective pressures (e.g. antimicrobial presence). In some cases, this association is very direct. The application of an antimicrobial treatment to control an infection caused by a virulent pathogen may lead to increased bacterial resistance. Both virulence and resistance can be disseminated by mobile genetic elements evolving susceptible strains to more pathogenic and resistant bacteria (Cepas and Soto 2020). The comparative study of clinical and non-clinical isolates might help to evidence this association.

The sampling frames of clinical and non-clinical isolates differ. Data on non-clinical isolates reported to the EU are collected from samples of healthy animals. These samples are collected randomly and are representative of the population in the country. The purpose of these samples is to assess the resistance level of the indicator bacteria in countries. On the other hand, data on clinical isolates are collected from samples of diseased animals. These samples are not randomly drawn from the population. Therefore, they are representative of the laboratories collecting samples but not necessarily representative of the population. The purpose of these samples is to identify the pathogen and a presumably successful medical treatment.

Bacterial exposure to antimicrobials promotes the emergence of resistance as these drugs remove drug-sensitive competitors selecting resistant bacteria (Read and Woods 2014). Global actions to address AMR are mainly focused on monitoring and reducing AMU in livestock and humans (Dadgostar 2019, World Health Organization (WHO) 2016). In Europe,

the European Surveillance of Veterinary Antimicrobial Consumption (ESVAC) collects harmonised AMU data from livestock as sales data in mg/Population Correction Unit (PCU) (European Medicines Agency (EMA) 2019). They are obtained by dividing the weight of the active ingredient sold for the use of an animal species/category in mg by the estimated average weight of this animal species/category. However, drugs are often licensed for more than one animal species/category. Therefore, these data provide only a general overview on AMU. Antimicrobial usage at farm-level, more accurate data, are also collected by some countries (Sanders et al. 2020).

Strategies to control and reduce AMR

Several strategies have been proposed in the literature to control and reduce AMR. Some of them are:

- Reduction of AMU in the human and animal sectors (McEwen and Collignon 2018, Li et al. 2017, World Health Organization (WHO) 2015a, Davies and Davies 2010).
- Discover new drugs that use new bacteria targets (Aslam et al. 2018).
- Combination of different antimicrobials by the use of different targets on the bacteria (Marquardt and Li 2018).
- Avoid substandard and falsified medical products (World Health Organization (WHO) 2017c).
- Increase health status by the use of preventive measures such as vaccination and hygienic procedures (Interagency Coordination Group on Antimicrobial Resistance (IACG) 2018, World Health Organization (WHO) 2017b, World Health Organization (WHO) 2015a).
- Genetic selection of livestock resistant to disease would reduce cases of disease and therefore, the use of antimicrobials.(Marquardt and Li 2018).
- Promote and support studies on alternative treatments to the use of antimicrobials such as plasmids (Buckner et al. 2018), peptides, phages, probiotics and vaccines (Aslam et al. 2018).
- Unify government efforts by (i) reducing the risk and uncertainty of antimicrobial clinical trials, (ii) boosting market value for not feeding animals antimicrobials, (iii) strengthen regulation of farm feeding, (iv) assuring the quality of antimicrobials and the prize of new and novel antimicrobials (Metz and Shlaes 2014).
- Promote pharmacokinetic and pharmacodynamic studies on toxicity and efficacy ranges of antimicrobials in order to provide recommendations for optimal use of drugs (Aminov 2017).

- Develop new and affordable resistance diagnostic tools that allow rapid identification of which drugs the disease-causing microorganisms are sensitive to.(Chan et al. 2020, Lee et al. 2020, Vasala et al. 2020, Aslam et al. 2018, World Health Organization (WHO) 2015a).
- Reduce as much as possible the AMR levels in breeding animals (Chuppava et al. 2018, Projahn et al. 2018). This will decrease the vertical transmission of AMR and, therefore, reduce the national AMR levels.
- Collect information by monitoring and surveillance systems on AMU and AMR in order to apply adequate interventions and monitor the impact on them (World Health Organization (WHO) 2015a).
- Increase the harmonisation level between surveillance and monitoring systems of AMU and AMR (Interagency Coordination Group on Antimicrobial Resistance (IACG) 2018, Simjee et al. 2018)

Laboratory and interpretation methods

There are different laboratory methods to test the phenotypic susceptibility of microorganisms to antimicrobials. They are mainly disk diffusion, micro broth dilution and automated methods such as VITEK®. The disk diffusion method consists of placing discs with a known antimicrobial concentration on a plate that has been previously inoculated with a microorganism. The inhibition halo, produced by the antimicrobial diffused through the agar of the plate, is measured in mm. The greater the inhibition halo, the greater the effectiveness of the antimicrobial against the microorganism (Hudzicki 2009). In the case of broth dilution, the process involves preparing two-fold dilutions of the antimicrobial agent. Starting with the most dilute solution, the microorganism growth is assessed by identifying the dilution in mg/ml where there is an inhibition of the microorganism growth (i.e. the Minimum Inhibition Concentration (MIC)). The automated systems create kinetic curves identifying the point of no growth of the microorganism providing also MICs. Substantial discrepancies were found in the results obtained by automated methods compared to the micro broth dilution method (Zhou et al. 2018). However, the VITEK 2® system, an automated method, seems to provide a relatively accurate assessment (Zhou et al. 2018, Bobenchik et al. 2015).

Quantitative AMR data (i.e. Inhibition zone diameters (IZD) based on the disk diffusion method or MICs based on the micro broth dilution method or automated methods) can be categorised applying an epidemiological or clinical approach, if the range of values tested include both cut-offs. Epidemiological cut-off values (ECOFFs) are preferred for monitoring and surveillance purposes differentiating the wild and non-wild type populations. In contrast, clinical breakpoints

(CBPs) define clinically a microorganism as susceptible, susceptible-increase exposure, or resistant depending on the probability of a therapeutic treatment succeeding (The European Committee on Antimicrobial Susceptibility Testing (EUCAST) 2020, European food Safety Authority (EFSA) 2019). There are many standards (The European Committee on Antimicrobial Susceptibility Testing (EUCAST), the Clinical & Laboratory Standards Institute (CLSI) or national standards among others) that define the CBPs and/or the ECOFFs.

The Whole Genome Sequencing (WGS) method allows microbial typing and AMR surveillance by specific allele profiles. WGS offers a higher level of data detail than traditional phenotypic methods for routine testing of AMR. However, this study is limited to bacterial typing by phenotypic patterns.

Conclusion

There is some evidence on the transmission of resistance between humans and animals (Lambrecht et al. 2019, Li et al. 2019, Ward et al. 2014, Mather et al. 2013, Spoor et al. 2013, Lowder et al. 2009). It is, therefore, necessary to assess the AMR issue from a multidisciplinary perspective, i.e. a One health approach, combining animal, human and environmental sectors. However, the literature describes a need for harmonisation in this field (Interagency Coordination Group on Antimicrobial Resistance (IACG) 2018, Schrijver et al. 2018, Simjee et al. 2018).

Against this background, we concluded that AMR is a complex, multidisciplinary and multifactorial phenomenon and AMU a main influencing factor on AMR. Therefore, we found necessary, as a baseline, to analyse the surveillance and monitoring systems on AMU and AMR in depth in Europe. We collected and assessed data on AMU and AMR of the human and livestock sectors together with foodborne AMR from six European countries. We found that data on non-clinical isolates from animals are harmonised. This is not the case for clinical isolates. This raises the question whether it would not be sufficient to collect data on non-clinical isolates. To this end, we investigated whether clinical and non-clinical isolates differed in resistance. If there were no differences, harmonisation between the two types of isolates would not be necessary. Therefore, studies were carried out to assess this issue.

Chapter 3: Monitoring Antimicrobial Resistance and Drug Usage in the Human and Livestock Sector and Foodborne Antimicrobial Resistance in Six European Countries

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ORIGINAL RESEARCH

Monitoring Antimicrobial Resistance and Drug Usage in the Human and Livestock Sector and Foodborne Antimicrobial Resistance in Six European Countries

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Infection and Drug Resistance

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Introduction: Antimicrobial resistance (AMR), associated with antimicrobial use (AMU), is a major public concern. Surveillance and monitoring systems are essential to assess and control the trends in AMU and AMR. However, differences in the surveillance and monitoring systems between countries and sectors make comparisons challenging. The purpose of this article is to describe all surveillance and monitoring systems for AMU and AMR in the human and livestock sectors, as well as national surveillance and monitoring systems for AMR in food, in six European countries (Spain, Germany, France, the Netherlands, the United Kingdom and Norway) as a baseline for developing suggestions to overcome current limitations in comparing AMU and AMR data.

Methods: A literature search in 2018 was performed to identify relevant peer-reviewed articles and national and European grey reports as well as AMU/AMR databases.

Results: Comparison of AMU and AMR systems across the six countries showed a lack of standardization and harmonization with different AMU data sources (prescription vs sales data) and units of AMU and AMR being used. The AMR data varied by sample type (clinical/non-clinical), laboratory method (disk diffusion, microdilution, and VITEK, among others), data type, ie quantitative (minimum inhibition concentration (MIC) in mg/L/inhibition zone (IZ) in mm) vs qualitative data (susceptible-intermediate-resistant (SIR)), the standards used (EUCAST/CLSI among others), and/or the evaluation criteria adopted (epidemiological or clinical).

Discussion: A One Health approach for AMU and AMR requires harmonization in various aspects between human, animal and food systems at national and international levels. Additionally, some overlap between systems of AMU and AMR has been encountered. Efforts should be made to improve standardization and harmonization and allow more meaningful analyses of AMR and AMU surveillance data under a One Health approach.

Keywords: AMR, AMU, food-producing animals, harmonization, monitoring, surveillance

Introduction

Antimicrobial use (AMU) in the last few decades is the main trigger for antimicrobial resistance (AMR) in humans and animals. For example, broad use of fluoroquinolones, effective antimicrobials against gram-positive and gram-negative bacteria, in humans and some animal populations has caused high resistance rates.[1] Antibiotics like colistin, that have issues with side effects but still have low resistance rates, have been reconsidered as a last-line drug due to a lack of alternative antimicrobials for multidrug-resistant Gram-negative bacteria.[2] This global threat includes both pathogenic and commensal bacteria. In order to tackle

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the AMR crisis, several global strategies have been developed such as the Global Action Plan (GAP) of the World Health Organization (WHO),[3] the new European One Health Action Plan against AMR[4] and the Central Asian and Eastern European Surveillance of Antimicrobial Resistance network (CAESAR).[5]

Surveillance[6] and monitoring[6] systems of AMU and AMR in humans and animals are essential to assess and subsequently control the global trends in the use of antimicrobials and antimicrobial susceptibility patterns of bacteria in different populations.[7] Using a One Health approach, zoonotic and indicator bacteria are of particular relevance.

Surveillance and monitoring systems are one of the five strategies of the GAP. However, even when the proper implementation of these systems enables the collection of reliable and good quality data, not all countries worldwide have surveillance and monitoring systems in place so it is not possible to perform a global comparison.

Several projects address the systems' evaluation of AMU and AMR in human, livestock and food sectors in Europe and also across European countries. As an illustration, the Ecology from Farm to Fork Of microbial drug Resistance and Transmission (EFFORT) project[8] is a relevant work collecting AMU and AMR data from broilers, pigs, turkeys, veal calves, rainbow trout and companion animals at farm level across different EU countries. Additionally, the Antibiotic Resistance Dynamics: the influence of geographic origin and management systems on resistance gene flows within humans, animals and the environment (ARDIG)[9] project gathers AMU and AMR data from the human and animal sectors together with AMR data collection from food at European level. Likewise, another crucial work carried out at global level and in the animal sector is the Network on quantification of veterinary Antimicrobial usage at herd level and Analysis, CommunicaTion and benchmarkING to improve responsible usage (AACTING).[10] The latter initiative has generated a review of existing systems that collect AMU data at farm level.

This report follows on the work carried out in the ARDIG project and provides a review of AMU and AMR surveillance and monitoring systems, adopting a One Health approach, currently available in six European countries that perform routine surveillance, as well as systems at a European level.

It will make recommendations regarding the harmonization of surveillance and monitoring systems across Europe with a view to help overcome current limitations in comparing AMU and AMR data captured by these systems from different sectors and countries within Europe.

Materials and Methods

In this manuscript, we gathered key features of surveillance and monitoring systems on AMU and AMR in livestock and humans as well as AMR systems in food from Spain, Germany, France, the Netherlands, Norway, the United Kingdom (UK) together with its regions and Europe between 2014 and 2017.

A literature search in 2018 was performed using PubMed to identify relevant peer-reviewed articles and the internet to identify national and European grey reports as well as AMU/AMR databases. The terms used for the search are “antimicrobial resistance”, “antimicrobial use”, “Spain”, “Germany”, “UK”, “United Kingdom”, “Scotland”, “Wales”, “England”, “Northern Ireland”, “Netherlands”, “France”, “Norway”, “Europe”, “food”, “human”, “animal”, “surveillance”, “system” and “monitoring”. Additionally, a questionnaire asking for detailed information on any available AMR and AMU database in each country was developed and sent to all collaborating institutes for completion (Supplementary materials).

A detailed systems' description by country and sector has been performed in order to detect and define the lack of harmonization and standardization on AMU and AMR.

Results

Antimicrobial Resistance Surveillance and Monitoring Systems

A general overview on AMR monitoring and surveillance systems is provided in Table 1. The variables collected in the table are the country/region, database name, data type, data origin, unit, interpretation standard, evaluation criteria, public data, published report, report language, submitting data to Europe, laboratory method and set-up year of the database. Additionally, Figures 1–3, showing AMR systems reporting and not reporting to EU per country and sector, are provided.

Europe

The European Food Safety Authority (EFSA)[11] is responsible for providing independent scientific advice and communication on food chain risks to risk managers and the public. EFSA together with the European Centre for Disease Control and Prevention (ECDC) collect annually AMR data on humans, food and healthy animals from the

Table 1 Features of AMR Databases in Human, Food and Animal Sectors by Region

Country/ Region	Data Type	Data Origin	Database	Unit	Interpretation Standard	Interpretation Approach	Public Report	Language of the Report	Communication to EU	Labor Method	Year System Developed
Germany	C	Clinical	ARS	MIC/SIR	EUCAST-CLSI-DIN	CBP	Interactive database: ARS (https://ars.rki.de/)	German	No	Several	2008
Germany	H	Clinical	ARS	MIC/SIR	EUCAST-CLSI-DIN	CBP	Interactive database: ARS (https://ars.rki.de/)	German	EARS-NET	Several	2008
Germany (Lower saxony)	C	Clinical	ARMIN	MIC/SIR	EUCAST-CLSI	CBP	Interactive report: ARMIN (https://www.nlga.niedersachsen.de/infektionsschutz/armin_resistenzentwicklung/armin_interaktiv/)	German	No	Several	2006
Germany (Lower saxony)	H	Clinical	ARMIN	MIC/SIR	EUCAST-CLSI	CBP	Interactive report: ARMIN (https://www.nlga.niedersachsen.de/infektionsschutz/armin_resistenzentwicklung/armin_interaktiv/)	German	No	Several	2006
Germany	H	Clinical	MRSA-KISS	SR	Not defined	CBP	MRSA-KISS Referenzdaten (https://www.nrz-hygiene.de/surveillance/kiss/mrsa-kiss/)	German	No	Several	2006
Germany	H	Clinical	SARI-KISS	SR	EUCAST-CLSI-DIN	CBP	SARI Resistenzdaten (https://eu-burden.info/sari/ab.php)	German	No	Several	2000
Germany	H	Clinical	ICU-KISS and OP-KISS	SR	Not defined	CBP	Referenzdaten (https://www.nrz-hygiene.de/surveillance/kiss/op-kiss/)	German	No	Several	1997
Germany	H/C	Clinical	PEG	MIC/SIR	EUCAST	CBP	Database: https://www.p-e-g.org/resistenz/database/ Reports: https://www.p-e-g.org/berichte-der-studien.html	German	No	Microdilution	1975
Germany	H/C	Clinical	BARDA	MIC/SIR	EUCAST	CBP	No	No	No	Microdilution	2019
Germany	A	Clinical	GERMVET	MIC	CLSI	CBP	GERMAP (http://www.p-e-g.org/econtext/germap) GERMVET (https://www.bvl.bund.de/SiteGlobals/Forms/Suche/Servicesuche_Formular.html?nn=1461338&resourceId=1412490&input_=10035804&pageLocale=de&templateQueryString=germ-vet&submit=Suchen)	English and German German	No	Microdilution	2001
Germany	A	Non-Clinical	ZOMO	MIC	EUCAST	ECOFFs	ZOMO (https://www.bvl.bund.de/DE/08_PresseInfothek/04_Publikationen/03_Berichte/infothek_berichte_node.html#doc1401838bodyText4)	German	EFSA	Microdilution	2009

(*Continued*)

Table 1 (Continued).

Country/ Region	Data Type	Data Origin	Database	Unit	Interpretation Standard	Interpretation Approach	Public Report	Language of the Report	Communication to EU	Labor Method	Year System Developed
Germany	F	Non-Clinical	ZOMO	MIC	EUCAST	ECOFFs	ZOMO (https://www.bvl.bund.de/DE/08_PresseInfothek/04_Publikationen/03_Berichte/infothek_berichte_node.html#doc1401838bodyText4)	German	EFSA	Microdilution	2009
Spain	H	Clinical	EARS-NET-ES (ISCIII)	MIC/SIR/IZ	EUCAST-CLSI	CBP	JIACRA Espana (http://www.resistenciaantibioticos.es/en/system/files/field/files/informe_jiacra-espana.pdf?file=1&type=node&id=410&force=0)	Spanish	EARS-NET	Several	1986
Spain	A	Clinical	VAV	MIC	EUCAST-CLSI	CBP	Report VAV (2005) (https://www.visavet.es/data/VAV2005.pdf)	Spanish and English	No	Disk diffusion/ Microdilution	1997
Spain	A	Non-Clinical	VAV	MIC	EUCAST	ECOFFs	JIACRA Espana (http://www.resistenciaantibioticos.es/en/system/files/field/files/informe_jiacra-espana.pdf?file=1&type=node&id=410&force=0) Report VAV (2005) (https://www.visavet.es/data/VAV2005.pdf) Simplified report on zoonoses and antimicrobial resistance of broilers and turkeys for poutry professionals (https://www.mapa.gob.es/ca/ganaderia/temas/sanidad-A-higiene-ganadera/sanidad-A/zoonosis-resistencias-antimicrobianas/resistencias_antimicrobianas.aspx) Simplified report on zoonoses and antimicrobial resistance of laying hens for professionals in the laying poultry sector (https://www.mapa.gob.es/ca/ganaderia/temas/sanidad-A-higiene-ganadera/sanidad-A/zoonosis-resistencias-antimicrobianas/resistencias_antimicrobianas.aspx) EFSA_Report (https://www.efsa.europa.eu/en/biological-hazards-data/reports)	Spanish Spanish and English Spanish Spanish English	EFSA	Disk diffusion/ Microdilution	1998

Spain	F	Non-Clinical	VAV	MIC	EUCAST	ECOFFs	Report VAV (2005) (https://www.visavet.es/data/VAV2005.pdf) Simplified report on zoonoses and antimicrobial resistance of broilers and turkeys for poutry professionals (https://www.mapa.gob.es/ca/ganaderia/temas/sanidad-A-higiene-ganadera/sanidad-A/zoonosis-resistencias-antimicrobianas/resistencias_antimicrobianas.aspx) Simplified report on zoonoses and antimicrobial resistance of laying hens for professionals in the laying poultry sector (https://www.mapa.gob.es/ca/ganaderia/temas/sanidad-A-higiene-ganadera/sanidad-A/zoonosis-resistencias-antimicrobianas/resistencias_antimicrobianas.aspx) EFSA_Report (https://www.efsa.europa.eu/en/biological-hazards-data/reports)	Spanish and English Spanish Spanish English	EFSA	Disk diffusion/ Microdilution	2000
England/ Northern Ireland	C	Clinical	SGSS	SIR	EUCAST-BSAC-CLSI	CBP	ESPAUR (https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/759975/ESPAUR_2018_report.pdf)	English	No	Several	2014
England/ Northern Ireland	H	Clinical	SGSS	SIR	EUCAST-BSAC-CLSI	CBP	ESPAUR (https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/759975/ESPAUR_2018_report.pdf)	English	EARS-NET	Several	2014
England/ Wales	A	Clinical	APHA VET PATHOGENS	SR/MIC (IZ)	BSAC (EUCAST)	CBP	UK-VARSS (https://www.gov.uk/government/collections/veterinary-antimicrobial-resistance-and-sales-surveillance)	English	No	Disk diffusion	1972
UK	A	Non-Clinical	EU HARMONIZED SURVEILLANCE	SIR/MIC	EUCAST	CBP/ECOFFs	UK-VARSS (https://www.gov.uk/government/collections/veterinary-antimicrobial-resistance-and-sales-surveillance) EFSA_Report (https://www.efsa.europa.eu/en/biological-hazards-data/reports)	English English	EFSA	Microdilution	2014

(*Continued*)

Table 1 (Continued).

Country/ Region	Data Type	Data Origin	Database	Unit	Interpretation Standard	Interpretation Approach	Public Report	Language of the Report	Communication to EU	Labor Method	Year System Developed
UK	F	Non-Clinical	EU HARMONIZED SURVEILLANCE	SIR/MIC	EUCAST	CBP/ECOFFs	UK-VARSS (https://www.gov.uk/government/collections/veterinary-antimicrobial-resistance-and-sales-surveillance) EFSA_Report (https://www.efsa.europa.eu/en/biological-hazards-data/reports)	English English	EFSA	Microdilution	2014
Scotland	H	Clinical	ECOSS	SIR	EUCAST	CBP	SOONAR (https://www.hps.scot.nhs.uk/web-resources-container/scottish-one-health-antimicrobial-use-and-antimicrobial-resistance-in-2017/)	English	No	Several	2013
Scotland	H	Clinical	ECOSS	SIR	EUCAST	CBP	SOONAR (https://www.hps.scot.nhs.uk/web-resources-container/scottish-one-health-antimicrobial-use-and-antimicrobial-resistance-in-2017/)	English	EARS-NET	Several	2013
Northern Ireland	A	Clinical	AFBI	SIR	CLSI	CBP	UK-VARSS (https://www.gov.uk/government/collections/veterinary-antimicrobial-resistance-and-sales-surveillance) All-Island Animal Disease Surveillance Report (https://www.afbini.gov.uk/publications/all-island-animal-disease-surveillance-report-2016)	English	No	Disk diffusion	2010
Scotland	A	Clinical	SRUC	SIR	BSAC	CBP	SOONAR (https://www.hps.scot.nhs.uk/web-resources-container/scottish-one-health-antimicrobial-use-and-antimicrobial-resistance-in-2017/)	English	No	BSAC disk diffusion	2016
Wales	C	Clinical	Datastore	SIR	EUCAST (2012)	CBP	Antibacterial Resistance in Wales (http://www.wales.nhs.uk/sitesplus/888/page/94136)	English	No	Several	1999
Wales	H	Clinical	Datastore	SIR	EUCAST (2012)	CBP	Antibacterial Resistance in Wales (http://www.wales.nhs.uk/sitesplus/888/page/94136)	English	EARS-NET	Several	1999
Northern Ireland	C	Clinical	CoSurv	SIR	EUCAST	CBP	Surveillance of Antimicrobial Use and Resistance in Northern Ireland (http://www.publichealth.hscni.net/sites/default/files/AMR_annual_report_final_0.pdf)	English	No	Not defined	2009

Northern Ireland	H	Clinical	CoSurv	SIR	EUCAST	CBP	Surveillance of Antimicrobial Use and Resistance in Northern Ireland (http://www.publichealth.hscni.net/sites/default/files/AMR_annual_report_final_0.pdf)	English	EARS-NET	Not defined	2009
UK and Ireland	C	Clinical	BSAC	MIC and SIR	EUCAST-BSAC	CBP	BSAC (http://www.bsacsurv.org/)	English	No	Disk diffusion	1999
UK and Ireland	H	Clinical	BSAC	MIC and SIR	EUCAST-BSAC	CBP	BSAC (http://www.bsacsurv.org/)	English	No	Disk diffusion	2001
Norway	C	Clinical	NORM	MIC	EUCAST/ NordicAST	CBP	NORM (https://unn.no/Documents/Kompetansetjenester,%20-sentre%20og%20fagr%C3%A5d/NORM%20-%20Norsk%20overv%C3%A5kingssystem%20for%20antibiotikaresistens%20hos%20mikrober/Rapporter/NORM_NORM-VET_2017.pdf)	English	No	Disk diffusion	2000
Norway	C	Clinical	MSIS	MIC	EUCAST	CBP	NORM (https://unn.no/Documents/Kompetansetjenester,%20-sentre%20og%20fagr%C3%A5d/NORM%20-%20Norsk%20overv%C3%A5kingssystem%20for%20antibiotikaresistens%20hos%20mikrober/Rapporter/NORM_NORM-VET_2017.pdf)	English	No	Microdilution	1977
Norway	H	Clinical	NORM	MIC	EUCAST/ NordicAST	CBP	NORM (https://unn.no/Documents/Kompetansetjenester,%20-sentre%20og%20fagr%C3%A5d/NORM%20-%20Norsk%20overv%C3%A5kingssystem%20for%20antibiotikaresistens%20hos%20mikrober/Rapporter/NORM_NORM-VET_2017.pdf) Interactive report: MSIS (http://www.MSIS.no/) Interactive database: https://norm-atlas.no/	English English and Norwegian Norwegian	EARS-NET	Disk diffusion	2000
Norway	H	Clinical	MSIS	MIC	EUCAST	CBP	NORM (https://unn.no/Documents/Kompetansetjenester,%20-sentre%20og%20fagr%C3%A5d/NORM%20-%20Norsk%20overv%C3%A5kingssystem%20for%20antibiotikaresistens%20hos%20mikrober/Rapporter/NORM_NORM-VET_2017.pdf) Interactive report: MSIS (http://www.MSIS.no/)	English English and Norwegian	EARS-NET	Microdilution	1977

(*Continued*)

Table 1 (Continued).

Country/ Region	Data Type	Data Origin	Database	Unit	Interpretation Standard	Interpretation Approach	Public Report	Language of the Report	Communication to EU	Labor Method	Year System Developed
Norway	A	Clinical	NORM-VET	MIC	EUCAST	ECOFFs	NORM-VET https://unn.no/Documents/Kompetansetjenester,%20-sentre%20og%20fagr%C3%A5d/NORM%20-%20Norsk%20overv%C3%A5kingssystem%20for%20antibiotikaresistens%20hos%20mikrober/Rapporter/NORM%20NORM-VET%202013.pdf	English	No	Microdilution	1999
Norway	A	Non-Clinical	NORMVET	MIC	EUCAST	ECOFFs	NORMVET (https://unn.no/Documents/Kompetansetjenester,%20-sentre%20og%20fagr%C3%A5d/NORM%20-%20Norsk%20overv%C3%A5kingssystem%20for%20antibiotikaresistens%20hos%20mikrober/Rapporter/NORM_NORM-VET_2017.pdf) EFSA_Report (https://www.efsa.europa.eu/en/biological-hazards-data/reports)	English English	EFSA	Microdilution	1999
Norway	F	Non-Clinical	NORMVET	MIC	EUCAST	ECOFFs	NORMVET (https://unn.no/Documents/Kompetansetjenester,%20-sentre%20og%20fagr%C3%A5d/NORM%20-%20Norsk%20overv%C3%A5kingssystem%20for%20antibiotikaresistens%20hos%20mikrober/Rapporter/NORM_NORM-VET_2017.pdf) EFSA_Report (https://www.efsa.europa.eu/en/biological-hazards-data/reports)	English English	EFSA	Microdilution method	2004
France	C/H	Clinical	ONERBA (taken over in 2019 by SPF and loaded in ConsoRes)	MIC, SIR, IZ	CA-SFM	CBP	ONERBA (http://onerba.org/publications/rapports-onerba/)	French and English	EARS-NET	Several	1997
France	H	Clinical	BMR-Raisin (taken over in 2019 by SPF and loaded in ConsoRes)	SIR	CA-SFM	CBP	BMR-RAISIN (https://www.santepubliquefrance.fr/recherche/#search=BMR%20RAISIN)	French	No	Several	2002
France	A	Clinical	RESAPATH	IZ/SIR	CA-SFM	CBP	RESAPATH (https://resapath.anses.fr/resapath_uploadfiles/files/Documents/2017_RESAPATH%20annual%20report.pdf) ONERBA (http://onerba.org/publications/rapports-onerba/)	English and French English and French	No	Disk diffusion	1982

France	A/F	Non-Clinical	ANSES	MIC	EUCAST	ECOFFs	EFSA_Report (https://www.efsa.europa.eu/en/biological-hazards-data/reports)	English	EFSA	Microdilution	2010
The Netherlands	C	Clinical	ISIS-AR	MIC/IZ/SIR	EUCAST	CBP	NETHMAP (https://www.rivm.nl/bibliotheek/rapporten/2019-0038.pdf) Interactive report: ISISweb (https://isis-web.nl/interactieve_rapporten/bezoekvraag/)	English Dutch	No	Several	2008
The Netherlands	H	Clinical	ISIS-AR	MIC/IZ/SIR	EUCAST	CBP	NETHMAP (https://www.rivm.nl/bibliotheek/rapporten/2019-0038.pdf) Interactive report: ISISweb (https://isis-web.nl/interactieve_rapporten/bezoekvraag/)	English Dutch	EARS-NET	Several	2008
The Netherlands	A	Clinical	MARAN	MIC	EUCAST	ECOFFs	MARAN (https://www.rivm.nl/bibliotheek/rapporten/2019-0038.pdf)	English	No	Microdilution	2014
The Netherlands	A	Non-Clinical	MARAN	MIC	EUCAST	ECOFFs	MARAN (https://www.rivm.nl/bibliotheek/rapporten/2019-0038.pdf) EFSA_Report (https://www.efsa.europa.eu/en/biological-hazards-data/reports)	English English	EFSA	Microdilution	1998
The Netherlands	F	Non-Clinical	MARAN	MIC	EUCAST	ECOFFs	MARAN (https://www.rivm.nl/bibliotheek/rapporten/2019-0038.pdf) EFSA_Report (https://www.efsa.europa.eu/en/biological-hazards-data/reports)	English English	EFSA	Microdilution	2005
Europe	H	Clinical	EARSNET	MIC/IZ/SIR	EUCAST	CBP	Surveillance of antimicrobial resistance in Europe (https://ecdc.europa.eu/en/publications-data/surveillance-antimicrobial-resistance-europe-2017)	English	EARSNET	Dilution test/diffusion test	1998
Europe	A	Clinical	VetPath	MIC	CLSI	CBP	No	No	No	Microdilution	1998
Europe	A	Non-Clinical	EFSA	MIC/SIR	EUCAST	ECOFFs	The European Union summary report on antimicrobial resistance in zoonotic and indicator bacteria from humans, animals and food (https://www.efsa.europa.eu/en/efsajournal/pub/5598) The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks (https://www.efsa.europa.eu/en/efsajournal/pub/5500)	English English	EFSA	Dilution test/diffusion test	2010

(*Continued*)

Table 1 (Continued).

Country/ Region	Data Type	Data Origin	Database	Unit	Interpretation Standard	Interpretation Approach	Public Report	Language of the Report	Communication to EU	Labor Method	Year System Developed
Europe	F	Non-Clinical	EFSA	MIC	EUCAST	ECOFFs	The European Union summary report on antimicrobial resistance in zoonotic and indicator bacteria from humans, animals and food (https://www.efsa.europa.eu/en/efsajournal/pub/5598) The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks (https://www.efsa.europa.eu/en/efsajournal/pub/5500)	English English	EFSA	Dilution test/ diffusion test	2010
Europe	F	Non-Clinical	EASSA	MIC	EUCAST-CLSI	CBP	No	No	No	Microdilution	1998

EU Member States (MS) and some associated countries. EFSA publishes "The European Union summary report on antimicrobial resistance in zoonotic and indicator bacteria from humans, animals and food".[12] EFSA also publishes annually the "Trends and sources of zoonoses and zoonotic agents in foodstuffs, animals and feeding stuffs"[13] report ("EFSA report" onwards) for those countries which have not published this information.

The European Animal Health Study Center (CEESA)[14] is a non-governmental organization financed by the veterinary pharmaceutical industry doing research on AMR. Two relevant CEESA subsystems for this review are the VetPath monitoring system and the European Antimicrobial Susceptibility Surveillance in Animals (EASSA). CEESA monitors the antimicrobial susceptibility of major disease-causing bacterial pathogens in food animals (VetPath), and of foodborne and commensal bacteria in food animals (EASSA).

The European Antimicrobial Resistance Surveillance Network (EARS-Net)[15] is an AMR surveillance network in accordance with the legislation[16] for Europe and for the European Economic Area members.[17] Through EARS-Net, the ECDC collects AMR data from the EU Member States and publishes the annual EARS-Net report "Surveillance of antimicrobial resistance in Europe" which presents resistance percentages and trends for key resistant bacteria. Data are based on blood and cerebrospinal fluid isolates from humans.[18]

The European Medicines Agency (EMA), EFSA and ECDC have produced two joint inter-agency antimicrobial consumption and resistance analysis (JIACRA) reports[19] attempting to compare antimicrobial use in animals and humans to AMR in the sectors and to assess potential effects of AMU and AMR in animals on the situation in humans.

Spain

The Spanish Veterinary Antimicrobial Resistance Surveillance Network (VAV)[14,20] was created to monitor AMR. It consists of three programs dealing with healthy animals, sick animals and with food and it is performed by the Ministry of Agriculture, Fisheries and Food (MAPAMA). VAV submits non-clinical data to the EFSA that are included in the annual EFSA reports.

The MAPAMA publishes the annual zoonoses and antimicrobial resistance report.[21] This report is based on the annual EFSA report and informs on zoonotic pathogens and diseases in animals, humans and food in addition to data on AMR in some zoonotic bacteria and indicator bacteria according to the EU legislation.[22,23]

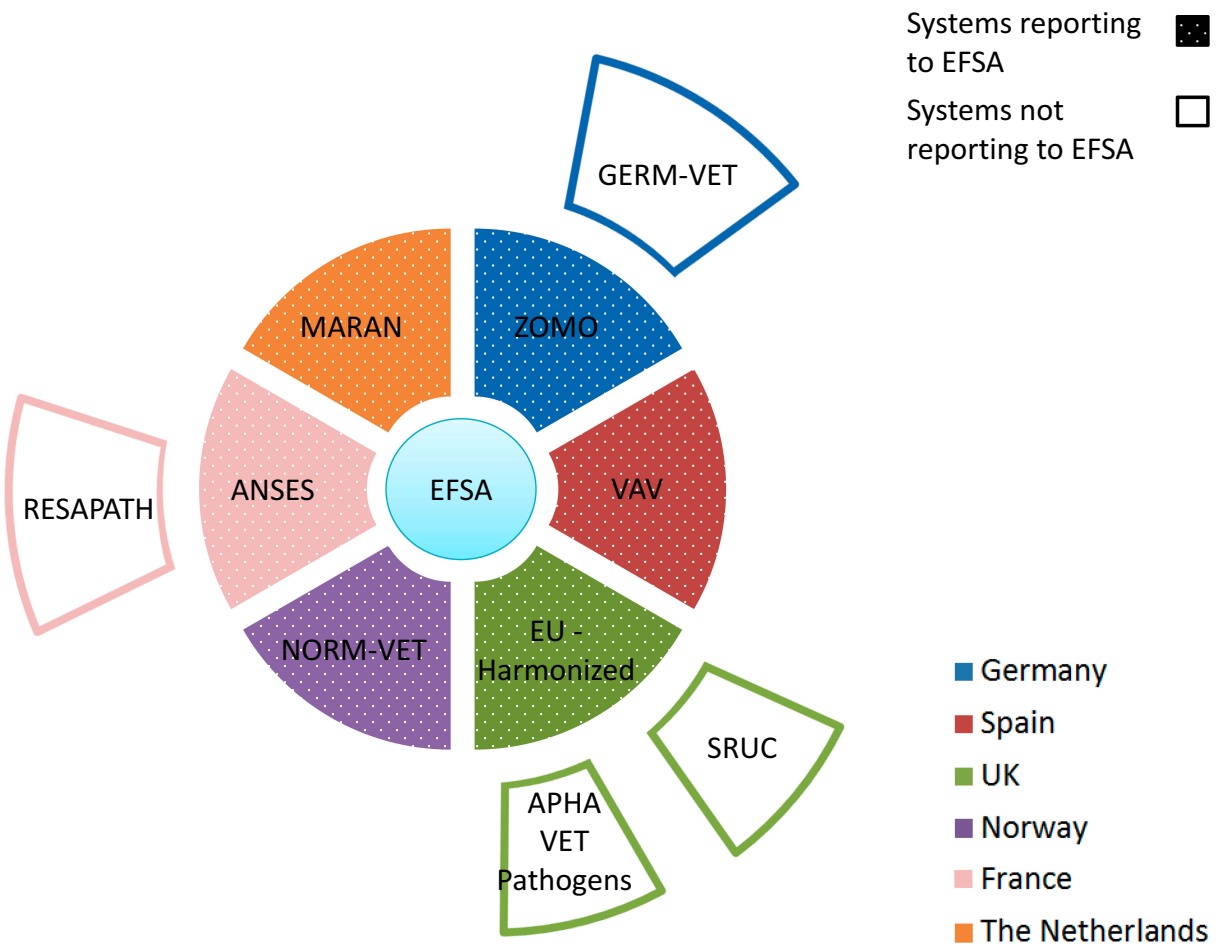

Figure 1 Overview on AMR systems in livestock in six European countries. Inner ring systems (dotted sections) report AMR data to EFSA while outer ring systems not. For details on the systems and their relationship, see the body of the text.

The Spanish national plan to tackle and reduce AMR (PRAN)[24] has been set up by the Spanish Agency for Consumer Affairs, Food Security and Nutrition (AECOSAN). PRAN publishes a simplified report on zoonoses and antimicrobial resistance of chickens and turkeys for poultry professionals and a simplified report on zoonoses and antimicrobial resistance of laying hens for professionals in the laying hen sector.[21] In addition, PRAN publishes the JIACRA Spain report[25] which assesses the relationship between AMU and AMR in humans and animals in Spain.

On the medical side, the national center of Carlos III Institute (ISCIII) coordinates and manages the national AMR database (EARS-Net-ES) submitting the data to the EARS-Net.[25]

The Netherlands

The AMR monitoring system on animals and food in the Netherlands is the "Monitoring of Antimicrobial Resistance and Antibiotic Usage in Animals in the Netherlands" (MARAN) bringing together the AMR food database of the Food and Consumer Product Safety Authority (NVWA).[26]

The Netherlands publishes data on the resistance of foodborne pathogens and of commensal indicators from livestock and food in the annual report also referred to as MARAN.[27] The report is produced in collaboration with the NVWA, the National Institute for Public Health and the Environment (RIVM), the Netherlands Veterinary Medicines Institute (SDa), the University of Utrecht and Wageningen University and Research.

In the human sector, the Infectious Disease Surveillance Information System on Antibiotic Resistance (ISIS-AR)[28,29] aims at monitoring AMR in major pathogens. The Dutch Foundation of the Working Party on Antibiotic Policy (SWAB) publishes the annual report "Consumption of antimicrobial agents and antimicrobial resistance among medically important bacteria in the Netherlands" (NethMap).[26]

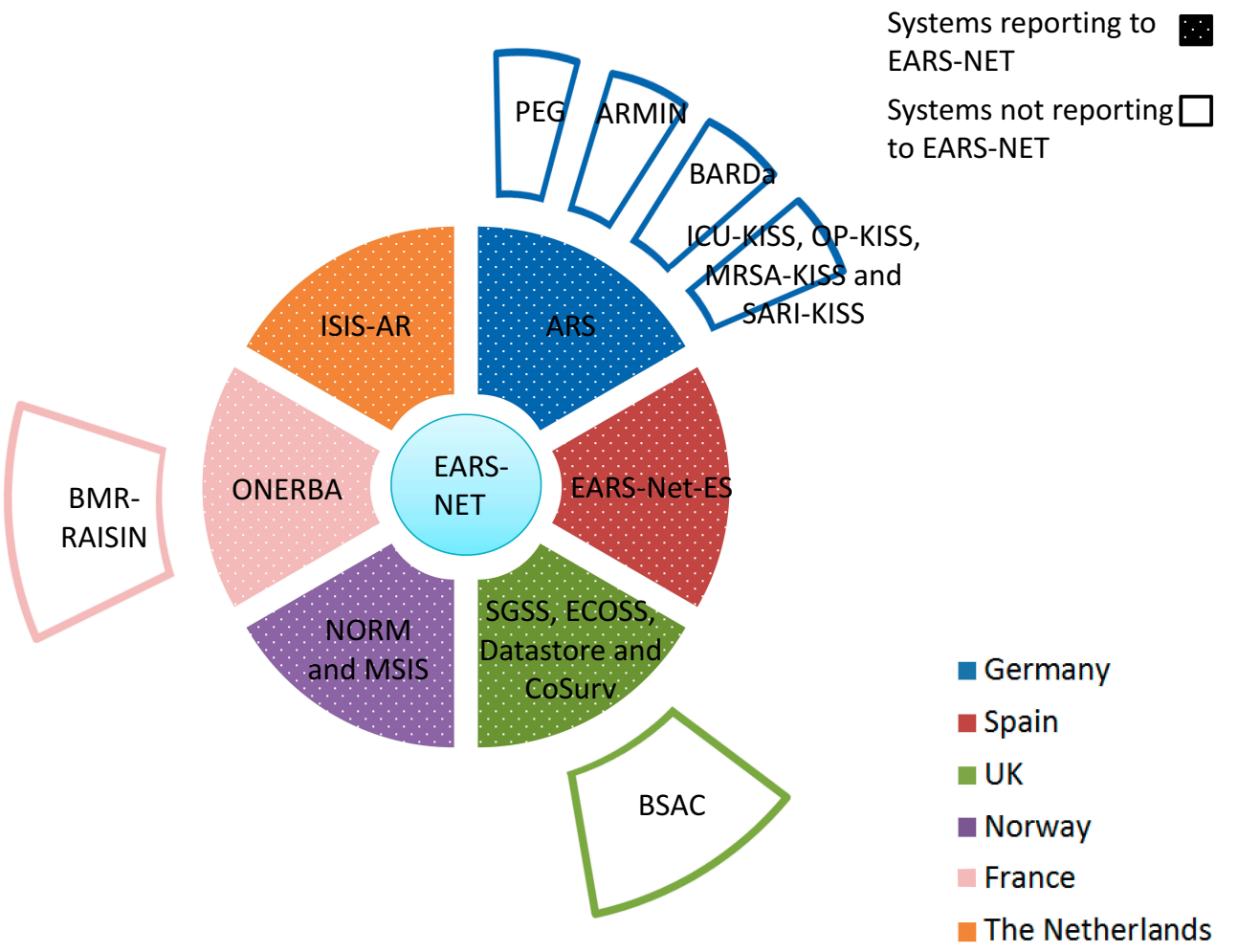

Figure 2 Overview on AMR systems in humans in six European countries. Inner ring systems report (dotted sections) AMR data to EARS-NET while outer ring systems not. For details on the systems and their relationship, see the body of the text.

This report provides resistance data for outpatients, inpatients and care in nursing homes. It reports on several surveillance programs such as the ISIS-AR and *Mycobacterium tuberculosis* surveillance program and others. It has been created by the Ministry of Health, Welfare and Sport and the Dutch Society of Medical Microbiology (NVMM) and coordinated by the RIVM.

United Kingdom

Several systems and reports coexist to monitor AMR in animals in the UK, which is based on the EU decision.[30] The EU harmonized surveillance system (a native UK system) collects mandatory AMR data on indicator commensal *Escherichia coli* and/or *Campylobacter* spp. from meat and faecal/caecal content of healthy animals (chicken, cattle/beef, turkey and pigs) in the UK. There are also UK National Control Programs for *Salmonella* in layers, broilers and turkeys, which are hosted in the EU harmonized surveillance system.

In England and Wales the scanning surveillance system Vet Pathogens APHA[31] provides AMR data from diseased animals provided for diagnostic services on a voluntary basis by veterinarians covering all relevant bacteria and animal species.

In Scotland, a surveillance system carried out by the Scotland's Rural College Veterinary Services and Capital Diagnostics (SRUC) collects clinical isolates from animals.

In Northern Ireland, an AMR surveillance system performed by the Agri-Food Biosciences Institute (AFBI) collects livestock clinical data from post-mortem investigation of colibacillosis or similar diseases. *E. coli* isolates mainly originate from samples coming from less than 2-week old calves and animals with bovine mastitis.[32]

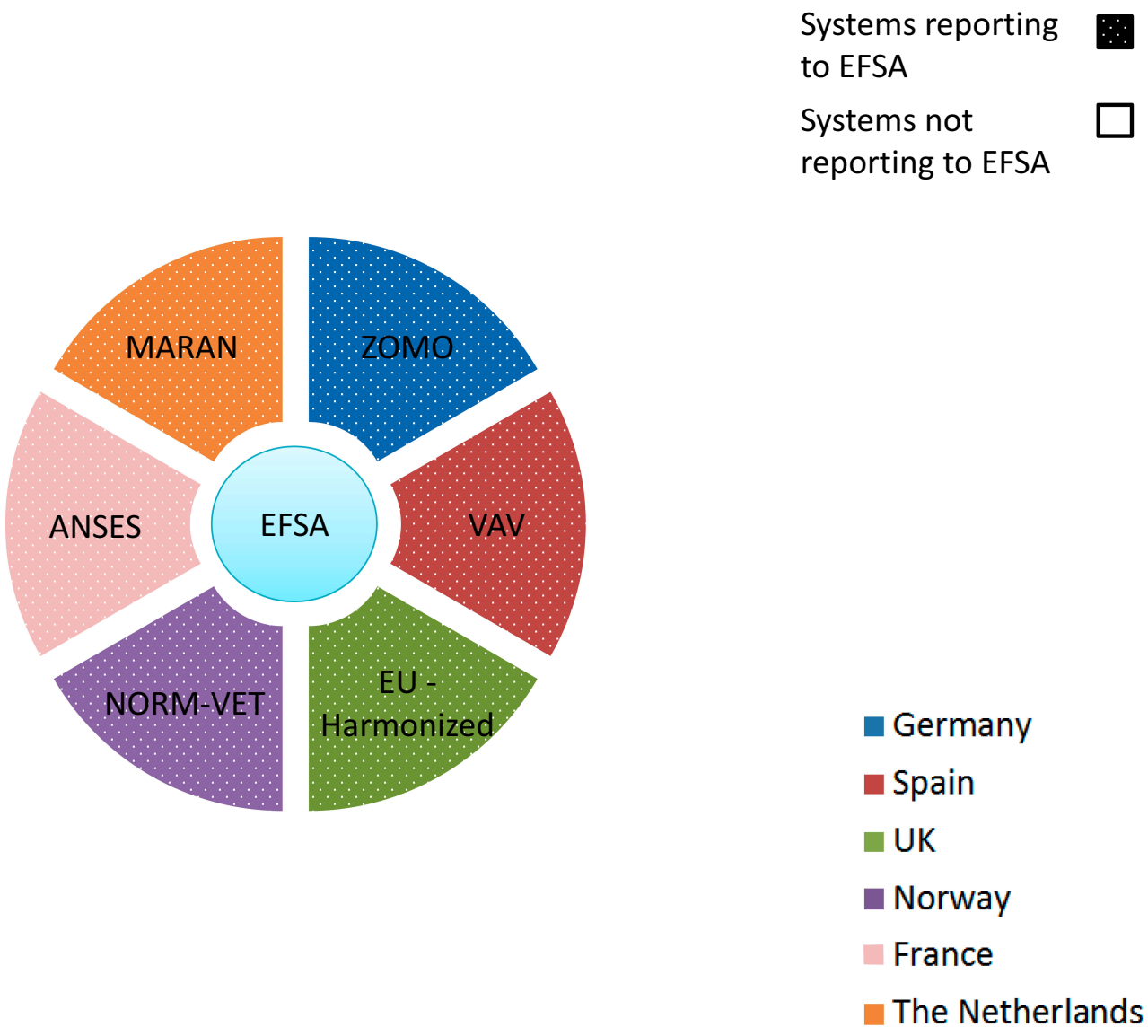

Figure 3 Overview on AMR systems in food in six European countries. Ring systems (dotted sections) report AMR data to EFSA. For details on the systems and their relationship, see the body of the text.

The annual report "UK-Veterinary Antibiotic Resistance and Sales Surveillance" (UK-VARSS) promoted by the UK government and produced by the Veterinary Medicine Directorate (VMD) provides details on veterinary AMR and AMU data in the UK.[32]

On the human side, the Resistance Surveillance Program of the British Society for Antimicrobial Chemotherapy (BSAC)[33] publishes antibiotic resistance data from participating laboratories in the UK and Ireland for a range of clinically significant bacteria from respiratory infections from the community (since 1999), hospitals (since 2008) and bloodstream infections (since 2001).

Public Health England's Second Generation Surveillance System (SGSS) captures routine laboratory surveillance data on infectious diseases and antimicrobial resistance from 98% of National Health Service (NHS) laboratories across England. SGSS data are reported annually in the English surveillance program for antimicrobial utilization and resistance (ESPAUR) report.[34,35]

The Electronic Communication of Surveillance in Scotland (ECOSS) database collects AMR data from participating NHS and reference laboratories in Scotland.[36] The data are published together with the AMR data on animals from the SRUC and several AMU data sources

from humans in the Scottish One Health Antimicrobial Use and Antimicrobial Resistance (SONAAR) report.[37]

The medical AMR data in Northern Ireland are collected on a voluntary basis by the CoSurv database. These data are published annually in the "Surveillance of Antimicrobial Use and Resistance in Northern Ireland" report ("NI report"). This report was published for the first time in 2017 by the Public Health Agency (PHA).

Finally, the DataStore is an open-access database that collects on a voluntary basis AMR data from Wales covering all hospital labs. The DataStore data are annually published in the "Antibacterial Resistance in Wales" report.

Norway

The Norwegian Surveillance System for Communicable Diseases (MSIS)[38] together with the Norwegian Surveillance System for Antimicrobial Drug Resistance (NORM) and Norwegian Veterinary Antimicrobial Resistance Monitoring (NORM-VET) system are the three AMR surveillance programs in Norway.[39] These systems publish their data in the "Usage of Antimicrobial Agents and Occurrence of Antimicrobial Resistance in Norway" (NORM/NORM-VET) report.[40] This annual report provides updated data on the occurrence and distribution of AMU and AMR in the human, animal and food sectors.

France

The French Agency for Food, Environmental and Occupational Health & Safety (ANSES) monitors AMR data associated with farming, food and the environment.[43] This institution coordinates the French surveillance network for antimicrobial resistance in pathogenic bacteria of animal origin (RESAPATH) and the Salmonella network. In addition, ANSES collects AMR non-clinical data from the national programs and the Salmonella network to be submitted to the EFSA.

The Salmonella network is a surveillance system set up to control non-human *Salmonella* throughout the food chain. Samples are collected from healthy animals, food and the environment.

The passive voluntary surveillance system RESAPATH provides in the annual RESAPATH report the AMR data compilation for the primary bacterial species and general isolates from sick animals from each animal sector.[41] This surveillance system started in 1982 under the name of RESABO (only for bovine species). In 2000, it was extended to pigs and poultry and in 2007 to other species including small ruminants, horses and companion animals. This network collaborates with the National Observatory of the Epidemiology of Bacterial Antibiotic Resistance (ONERBA).

On the medical side, ONERBA is the annual French report on AMU and AMR as well as the main AMR network collecting data from a complex network of subsystems. Currently (2019), the French health system (SPF) is taking over the ONERBA network and results are reported in the new tool ConsoRes.[42] This tool has been set up by the support centers for the prevention of healthcare-associated infections (CPias) Great East and New Aquitaine. Additionally, the AMR community network driven by SPF reports results using the Medqual[43] tool coordinated by the CPias Pays de la Loire. Finally, the Alert, Investigation and Surveillance of Nosocomial Infection Network (RAISIN)[44,45] coordinates nationally the nosocomial infection surveillance coordination centers (CCLIN), now CPias. The RAISIN network includes several surveillance system modules. The private RAISIN module for multi-drug resistant bacteria BMR-RAISIN reports on AMR data in the community. However, it will be replaced shortly by the tool ConsoRes (2019).[46]

Germany

The German veterinary monitoring system (GERM-VET) collects clinical AMR data in Germany from companion and food-producing animals. These data are published in detail by the Federal Office of Consumer Protection and Food Safety (BVL) in a report with the same denomination.[47]

AMR-testing in the Zoonosis-Monitoring system (ZOMO) is carried out by the Federal Institute for Risk Assessment (BfR). The results are published in the annual zoonosis monitoring report by the BVL.[48] The report contains data about zoonotic and commensal bacteria in diverse food chains that are also reported to the EFSA together with AMR-data on Salmonella from the national control programs.

Antimicrobial Resistance Surveillance (ARS)[49] is the national AMR surveillance system in human medicine. Established by the Robert Koch Institute, it collects routine susceptibility data for all bacterial species from any kind of sample site from hospital care as well as from outpatient care institutions by an increasing number of laboratories participating on a voluntary basis. Results for main pathogens are published via an interactive database on the ARS website.

Besides the national surveillance ARS, the federal state Lower Saxony sets up a similar system (ARMIN).[50] A further system has recently been set up in Bavaria (BARDa).[51]

The Hospital Infection Surveillance System (KISS) is the nosocomial infection surveillance system in hospitals formed by several sub-systems collecting AMU and AMR data.[52] This network assimilated the Surveillance of Antimicrobial Use and Bacterial Resistance in Intensive Care Units (SARI).[53,54] SARI collected on a voluntary basis aggregated data on antimicrobial sensitivity for selected pathogenic bacteria and AMU-AMR development. The project is organized by the Institute for Hygiene and Environmental Medicine of the Charité, Berlin.[55] Patient-based and unit-based AMR data (MRSA, VRE, ESBL) are collected as well.

Finally, the Paul Ehrlich Society for Chemotherapy (PEG)[56] is a society that conducts studies on antimicrobial resistance in human pathogens as part of a longitudinal study in both hospital and community sectors. Results are presented as an interactive database on the PEG website. The report on Antibiotic Consumption and the Spread of Antibiotic Resistance in Human and Veterinary Medicine in Germany (GERMAP),[55] a joint work of the PEG, the BVL and Infectiology Freiburg, is published on a regular basis. This report provides AMU and AMR data and trends in human and veterinary medicine in Germany since 2008. GERMAP publishes AMR-data mainly from GERM-VET, ARS and data of the PEG. The report publishes antibiotic consumption data from the community analyzed by the Research Institute of the largest German public non-private Health Insurance AOK (WIdO).

Antimicrobial Consumption Surveillance and Monitoring Systems

A general overview on AMU monitoring and surveillance systems is provided in Table 2. The variables collected in the AMU table are country/region, database name, data origin, unit, public data published report, report language, data source, submitting data to Europe and set-up year of the database. Additionally, Figures 4 and 5 show AMU systems reporting and not reporting to EU per country and sector.

The term "prescription data" has been used in the veterinary field in the later table as "usage data" covering what is prescribed by the veterinarian, supplied by the veterinarian under veterinary prescription, or administered by the farmer under veterinary prescription.

Europe

EMA monitors overall AMU in livestock through sales data in the European Surveillance of Veterinary Antimicrobial Consumption (ESVAC) project.[57,58] Since 2010, AMU is provided as an overall consumption (ie overall sales corrected for the animal population present in the country) in mg/kg. Since 2019, European countries are required to set up a data collection system in order to provide antibiotic consumption per species to ESVAC from 2022 onwards.

The Network on quantification of veterinary Antimicrobial usage at herd level and Analysis, CommunicaTion and benchmarkING to improve responsible usage (AACTING) provides in its website guidelines and information on farm level AMU collection systems from AACTING members, mostly European countries.[10]

Similar to ESVAC, on the medical side, European AMU data from the community and hospital sector are collected by the European Surveillance of Antimicrobial Consumption Network (ESAC-Net),[59] that is coordinated by ECDC. AMU data are collected by MS in the community and the hospital sector or both (total care). In addition, ECDC coordinates the Healthcare-Associated Infections Surveillance Network (HAI-Net) since the coordination of the Improving Patient Safety in Europe network (IPSE) was transferred to ECDC in 2008. The HAI-Net supports MS in the prevention and control of healthcare-associated infections (HAI) and coordinates the European point prevalence survey of HAI and AMU in acute care hospitals, the European surveillance of surgical site infections (HAI-Net SSI), the European surveillance of Healthcare-Associated Infections in intensive care units and the repeated prevalence surveys of HAI and AMU in European long-term care facilities.

Spain

The ESVAC-ES is a project from PRAN and carried out by the Spanish Agency of Medicaments and Sanitary Products (AEMPS).[60] It collects animal AMU data on a voluntary basis and reports annually these sales data from the veterinary sector to ESVAC. Since 2019, ESVAC-ES additionally collects prescription data. Moreover, since 2016, several collaboration programs between PRAN, MAPAMA and the animal production sectors have been implemented. In these programs that

Table 2 Features of AMU Databases in Human and Animal Sectors by Region

Country/ Region	Data type	Database	Unit	Public Report	Language of the Report	Communication to EU	Data Source	Year System Developed
Germany	C	WIdO	DDD/1000 insured day	GERMAP (http://www.p-e-g.org/econtext/GERMAP)	German and English	ESAC-Net	Reimbursement	1980
Germany	H	AVS	DDD/100 patient day and RDD/100 patient day	Interactive report: AVS-report (https://avs.rki.de/Content/ReferenceData/AIReport.aspx)	German	No	Prescription (hospital pharmacy)	2008
Germany	H	ADKA-IF-DGI	RDD/100 care day	ADKA-if_DGI Antiinfektiva-Surveillance (http://www.antiinfektiva-surveillance.de/files/kvr_2014-2015_adka-if-dgi_121116_v.4_open_access_geschwaerzt_neu.pdf)	German	No	Prescription (hospital pharmacy)	2015
Germany	H	SARI-KISS	DDD/1000 patients day and DDD	SARI-Antibiotikadaten (https://eu-burden.info/sari/ab.php)	German	No	Prescription (hospital pharmacy)	2000
Germany	A	DIMDI	Weight of active ingredient	GERMAP (http://www.p-e-g.org/econtext/GERMAP) DIMDI (https://www.bvl.bund.de/DE/08_PresseInfothek/01_FuerJournalisten_Presse/01_Pressemitteilungen/05_Tierarzneimittel/2010/2010_11_18_pi_abgabemengenregister.html;jsessionid=9D2FE13408BFF8D5F5E702CD3A473318.1_cid332)	German and English/ German	ESVAC	Sales (wholesalers)	2011
Germany	A	HIT	Therapy frequency	BVL (https://www.bvl.bund.de/DE/05_Tierarzneimittel/03_Tieraerzte/04_Therapiehaeufigkeit/Therapiehaeufigkeit_node.html)	German	No	Prescription	2014
Germany	A	QS	Therapy frequency	No	German	No	Prescription	2012
Germany	A	VetCab	Therapy frequency	No	No	No	Prescription	2007
Spain	A	Plan REDUCE	mg/PCU	Plan REDUCE (http://www.resistenciaantibioticos.es/es/system/files/field/files/primer_informe_programa_reduce_colistina_0.pdf?file=1&type=node&id=387&force=0)	Spanish	ESVAC since 2019	Prescription	Since 2016

Spain	C	MSCBS	DDD/ 1000 inhabitants day	JIACRA Espana (http://www.resistenciaantibioticos.es/en/system/files/field/files/informe_jiacra-espana.pdf?file=1&type=node&id=410&force=0) Interactive report: PRAN (http://www.resistenciaantibioticos.es/es/profesionales/vigilancia/mapas-de-consumo)	Spanish Spanish	ESAC-Net	Reimbursement	1978
Spain	H	IQVIA	DDD/ 1000 inhabitants day	JIACRA Espana (http://www.resistenciaantibioticos.es/en/system/files/field/files/informe_jiacra-espana.pdf?file=1&type=node&id=410&force=0) Interactive report: PRAN (http://www.resistenciaantibioticos.es/es/profesionales/vigilancia/mapas-de-consumo)	Spanish Spanish	ESAC-Net	Sales (hospital pharmacy)	2012
Spain	C	IQVIA	DDD/ 1000 inhabitants day	JIACRA Espana (http://www.resistenciaantibioticos.es/en/system/files/field/files/informe_jiacra-espana.pdf?file=1&type=node&id=410&force=0) Interactive report: PRAN (http://www.resistenciaantibioticos.es/es/profesionales/vigilancia/mapas-de-consumo)	Spanish Spanish	ESAC-Net	Sales (pharmacy)	2014
Spain	A	ESVAC-ES	Weight of active ingredient	JIACRA Espana (http://www.resistenciaantibioticos.es/en/system/files/field/files/informe_jiacra-espana.pdf?file=1&type=node&id=410&force=0)	Spanish	ESVAC	Sales (since 2019 prescription data also available)	2010
England	C	NHS BSA (PHE Antibiotic Prescribing Data Warehouse)	DDD/ 1000 inhabitants day and DDD/admissions year	ESPAUR (https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/759975/ESPAUR_2018_report.pdf)	English	ESAC-Net	Reimbursement	2014
England	H	IQVIA	DDD/1000 inhabitants day	ESPAUR (https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/759975/ESPAUR_2018_report.pdf)	English	ESAC-Net	Sales (hospital pharmacy)	2014
Scotland	C	PIS	DDD/ 1000 inhabitants day and items/1000 inhabitants day	Scottish One Health Antimicrobial Use and Antimicrobial Resistance (https://www.hps.scot.nhs.uk/resourcedocument.aspx?id=6971)	English	ESAC-Net	Reimbursement	1993

(*Continued*)

Table 2 (Continued).

Country/ Region	Data type	Database	Unit	Public Report	Language of the Report	Communication to EU	Data Source	Year System Developed
Scotland	H	HMUD	DDD/1000 occupied beds/day and DDD/ 1000 inhabitants day	Scottish One Health Antimicrobial Use and Antimicrobial Resistance (https://www.hps.scot.nhs.uk/resourcedocument.aspx?id=6971)	English	ESAC-Net	Reimbursement	2007
Northern Ireland	C	Electronic Prescribing Database	DDD/1000 beds/day, DDD/ 1000 inhabitants day and DDD/1000 admissions year	Surveillance of Antimicrobial use and Resistance in Northern Ireland (http://www.publichealth.hscni.net/sites/default/files/AMR_annual_report_final_0.pdf)	English	ESAC-Net	Reimbursement	2014
Northern Ireland	H	JAC Medicines Management Systems	DDD/1000beds day, DDD/ 1000 inhabitants day and DDD/1000 admissions year	Surveillance of Antimicrobial use and Resistance in Northern Ireland (http://www.publichealth.hscni.net/sites/default/files/AMR_annual_report_final_0.pdf)	English	ESAC-Net	Reimbursement	2014
Wales	C	Prescribing Information Data Warehouse (PSU)	Items/1000 patients year and items/1000 STAR-PU	Antimicrobial Usage in Primary Care in Wales (http://www.wales.nhs.uk/sitesplus/888/page/94136)	English	ESAC-Net	Reimbursement	2000
Wales	H	Medusa database	DDD/1000 beds day and DDD/1000 admissions year	Antimicrobial Usage in Secondary Care in Wales (http://www.wales.nhs.uk/sitesplus/888/page/94136)	English	ESAC-Net	Reimbursement	1995
UK	A	VMD	Weight of active ingredient and mg/kg	UKVARSS (https://www.gov.uk/government/collections/veterinary-antimicrobial-resistance-and-sales-surveillance)	English	ESVAC	Sales	1989
UK	A	BEIC	Weight of active ingredient	UKVARSS (https://www.gov.uk/government/collections/veterinary-antimicrobial-resistance-and-sales-surveillance)	English	No	Prescription	1986
UK	A	BPC stewardship	Weight of active ingredient	UKVARSS (https://www.gov.uk/government/collections/veterinary-antimicrobial-resistance-and-sales-surveillance)	English	No	Prescription	2012

UK	A	eMB-pigs	Weight of active ingredient and mg/kg	UKVARSS (https://www.gov.uk/government/collections/veterinary-antimicrobial-resistance-and-sales-surveillance)	English	No	Prescription	2016
UK	A	eMB-Cattle and Sheep	To be determined	eMB-Cattle and Sheep (http://beefandlamb.ahdb.org.uk/wp-content/uploads/2018/10/CHAWG-Fourth-Report-2018.pdf)	Englsih	No	Prescription	2018
UK	A	NML	mg/PCU, mg/kg, DDD-vet, DDD-vetUK,DCD-vet and DCD-vetUK	NML (https://www.nationalmilklaboratories.co.uk/vets/farm-assist)	No	No	Prescription	2017
Great Britain	A	Farmvet Systems	Weight of active ingredient and mg/kg	UKVARSS (https://www.gov.uk/government/collections/veterinary-antimicrobial-resistance-and-sales-surveillance)	English	No	Prescription	2015
Norway	A	Norwegian Prescription database (NorPD)	DDD	The Norwegian Prescription Database report (https://www.fhi.no/en/hn/health-registries/norpd/)	English	No	Prescription	2004
Norway	C	Norwegian Prescription database (NorPD)	DDD/1000 inhabitants day	NORM (https://unn.no/Documents/Kompetansetjenester,%20-sentre%20og%20fagr%C3%A5d/NORM%20-%20Norsk%20overv%C3%A5kingssystem%20for%20antibiotikaresistens%20hos%20mikrober/Rapporter/NORM_NORM-VET_2017.pdf) Norwegian Prescrition Database report (https://www.fhi.no/en/hn/health-registries/norpd/)	English English	ESAC-Net (only ambulatory)	Reimbursement	2004
Norway	H	Norwegian Prescription database (NorPD)	DDD/1000 inhabitants day	NORM (https://unn.no/Documents/Kompetansetjenester,%20-sentre%20og%20fagr%C3%A5d/NORM%20-%20Norsk%20overv%C3%A5kingssystem%20for%20antibiotikaresistens%20hos%20mikrober/Rapporter/NORM_NORM-VET_2017.pdf) Norwegian Prescrition Database report (https://www.fhi.no/en/hn/health-registries/norpd/)	English English	ESAC-Net (only ambulatory)	Reimbursement	2004

(*Continued*)

Table 2 (Continued).

Country/ Region	Data type	Database	Unit	Public Report	Language of the Report	Communication to EU	Data Source	Year System Developed
Norway	C/H	Norwegian drug wholesales statistics database (NIPH)	DDD/1000 inhabitants day	NORM (https://unn.no/Documents/Kompetansetjenester,%20-sentre%20og%20fagr%C3%A5d/NORM%20-%20Norsk%20overv%C3%A5kingssystem%20for%20antibiotikaresistens%20hos%20mikrober/Rapporter/NORM_NORM-VET_2017.pdf) Drug Consuption in Norway report (https://www.fhi.no/en/publ/2018/legemiddelstatistikk-20182-reseptregisteret-20132017/)	English English and Norwegian	No	Sales (wholesalers)	1970
Norway	H	Hospital pharmacies drug statistics database (NorPD)	DDD/1000 inhabitants day, DDD/ 100 beds days and DDD/ admissions year	NORM (https://unn.no/Documents/Kompetansetjenester,%20-sentre%20og%20fagr%C3%A5d/NORM%20-%20Norsk%20overv%C3%A5kingssystem%20for%20antibiotikaresistens%20hos%20mikrober/Rapporter/NORM_NORM-VET_2017.pdf)	English	ESAC-Net	Sales (hospital pharmacy)	2006
Norway	A	NORM-VET	Weight of active ingredient	NORMVET (https://unn.no/Documents/Kompetansetjenester,%20-sentre%20og%20fagr%C3%A5d/NORM%20-%20Norsk%20overv%C3%A5kingssystem%20for%20antibiotikaresistens%20hos%20mikrober/Rapporter/NORM_NORM-VET_2017.pdf)	English	ESVAC	Sales	1999
Norway	A	VetReg	Weight of active ingredient	NORMVET (https://unn.no/Documents/Kompetansetjenester,%20-sentre%20og%20fagr%C3%A5d/NORM%20-%20Norsk%20overv%C3%A5kingssystem%20for%20antibiotikaresistens%20hos%20mikrober/Rapporter/NORM_NORM-VET_2017.pdf)	English	No	Prescription	2011
Norway	H	NOIS	Weight of active ingredient and boxes	NOIS (https://www.fhi.no/hn/helseregistre-og-registre/nois/)	Norwegian	HAI-Net SSI	Prescription (hospital pharmacy)	2005

France	C	SNDS-SNIIRAM (taken over in 2019 by SPF and loaded in ConsoRes)	Number of boxes, number of tablets and concentration	No	No	No	Prescription (hospital pharmacy)	2003
France	C	SNDS-SNIIRAM (taken over in 2019 by SPF and loaded in ConsoRes)	Number of boxes, number of tablets and concentration	No	No	No	Reimbursement	2003
France	C	ANSM	DDD/ 1000 inhabitants day	Antibiotic consumption trends in France (https://ansm.sante.fr/S-informer/Points-d-information-Points-d-information/Evolution-des-consommations-d-antibiotiques-en-France-entre-2000-et-2015-Point-d-Information) Antibiotic consumption in France in 2016 (https://www.google.com/url?sa=t&rct=j&q=&esrc=s&source=web&cd=5&ved=2ahUKEwiCr5S-pMDjAhXIQkEAHWuRDFQQFjAEegQIBBAC&url=https%3A%2F%2Fwww.ansm.sante.fr%2Fcontent%2Fdownload%2F113089%2F1432671%2Fversion%2F1%2Ffile%2FRapport%2Bantibio_nov2017.pdf&usg=AOvVaw1dqdemy8MEH3MrXCwBPHbK)	French French	ESAC-Net	Sales (Pharmacy)	1999

(*Continued*)

Table 2 (Continued).

Country/ Region	Data type	Database	Unit	Public Report	Language of the Report	Communication to EU	Data Source	Year System Developed
France	H	ANSM	DDD/ 1000 inhabitants day	Antibiotic consumption trends in France (https://ansm.sante.fr/S-informer/Points-d-information-Points-d-information/Evolution-des-consommations-d-antibiotiques-en-France-entre-2000-et-2015-Point-d-Information) Antibiotic consumption in France in 2016 (https://www.google.com/url?sa=t&rct=j&q=&esrc=s&source=web&cd=5&ved=2ahUKEwiCr5S-pMDjAhXIQkEAHWuRDFQQFjAEegQIBBAC&url=https%3A%2F%2Fwww.ansm.sante.fr%2Fcontent%2Fdownload%2F113089%2F1432671%2Fversion%2F1%2Ffile%2FRapport%2Bantibio_nov2017.pdf&usg=AOvVaw1dqdemy8MEH3MrXCwBPHbK)	French French	ESAC-Net	Sales (Pharmacy)	1999
France	H	ATB-RAISIN (taken over in 2019 by SPF and loaded in ConsoRes)	DDD/1000 inpatient day	Surveillance de la consommation des antibiotiques (https://www.santepubliquefrance.fr/recherche/#search=ATB%20RAISIN)	French	No	Prescription (hospital pharmacy)	2001
France	A	INAPORC	DDD and DCD based on national SPC; DDDvet, DCDvet from EMA	No	No	No	Prescription	2010
France	A	Permanent Observatory of Antibiotics in Veal Calf Farms	The number of antimicrobial treatment per calf and batch, the number of antimicrobial treatment days per calf, the total quantity of active ingredient per calf and the Animal Level of Exposure to Antimicrobials (ALEA)	No	No	No	Prescription	2016

France	A	GVET	The number of antimicrobial treatment, the number of antimicrobial treatment days, UDD, UCD, DDD, DCD, DDDvet and DCDvet	No	No	No	Prescription	2017
France	A	ANMV	ADDkg in tonnes, ACDkg, ALEA	Sales survey of veterinary medicinal products containing antimicrobials in France (https://www.anses.fr/en/system/files/ANMV-Ra-Antibiotiques2017EN.pdf)	English and French	ESVAC	Sales (Pharmacy)	1999
The Netherlands	C	SFK	DDD/ 1000 inhabitants day and DDD	Nethmap (https://www.rivm.nl/bibliotheek/rapporten/2019-0038.pdf)	English	ESAC-Net	Sales (Pharmacy)	1990
The Netherlands	C	SNIV	DDD/1000 residents day	Nethmap (https://www.rivm.nl/bibliotheek/rapporten/2019-0038.pdf)	English	No	Sales (Pharmacy)	2007
The Netherlands	A	FIDIN	Weight of active ingredient	MARAN (https://www.rivm.nl/bibliotheek/rapporten/2019-0038.pdf)	English	ESVAC (since 2009)	Sales	1999
The Netherlands	A	SDa	Weight of active ingredient, DDDvet and DDDA NAT	MARAN (https://www.rivm.nl/bibliotheek/rapporten/2019-0038.pdf) SDa report (https://www.autoriteitdiergeneesmiddelen.nl/en/publications)	English English and Dutch	No	Prescription	2010
The Netherlands	H	Dutch hospital electronic prescribing system (SWAB)	DDD/100 patient day and DDD/ 1000 inhabitants day	Nethmap (https://www.rivm.nl/bibliotheek/rapporten/2019-0038.pdf)	English	ESAC-Net	Sales (hospital pharmacy)	1996
The Netherlands	A	MediRund	DDD/animal/year	No	No	No	Prescription	2012

(*Continued*)

Table 2 (Continued).

Country/ Region	Data type	Database	Unit	Public Report	Language of the Report	Communication to EU	Data Source	Year System Developed
Europe	C/H	ESAC-Net	DDD/ 1000 inhabitants day	Antimicrobial consumption - Annual Epidemiological Report (https://ecdc.europa.eu/en/antimicrobial-consumption/surveillance-and-disease-data/report-protocol)	English	ESAC-Net	Sales and reimbursement	2002
Europe	A	ESVAC	mg/PCU	ESVAC (http://www.ema.europa.eu/ema/index.jsp?curl=pages/regulation/document_listing/document_listing_000302.jsp)	English	ESVAC	Sales	2010

are called "Plan REDUCE" the animal sectors provide AMU data on a voluntary basis to PRAN. These data together with the prescription data that ESVAC-ES collects will be submitted to ESVAC.

On the medical side, the Ministry of Health, Consumption and Social Welfare (MSCBS) database collects community reimbursement data on antimicrobials dispensed from only official prescriptions in the public system. The system is run by the General Directorate of Basic Services of the National Health and Pharmacy System.[61] The database for Pharmacoepidemiological Research in Primary Care (BIFAP) and the Primary Care Clinical Database (BDCAP) provide primary care data integrated into the MSCBS database. Spain is able to provide primary and secondary care sales data through The Human Data Science Company (IQVIA) database, formerly Quintiles and IMS Health.[24,62]

The PRAN website provides charts based on data collected from the latter databases with estimates on AMU in hospitals since 2012 and in the community (national and regional) since 2014.[63]

The Netherlands

The MARAN report includes AMU data from two sources[26]:

- The Federation of the Dutch veterinary pharmaceutical industry (FIDIN) provides antibiotic sales data on the major livestock farming sectors.[27]
- The Netherlands Veterinary Medicines Institute (SDa) is an independent institute that promotes responsible drug consumption. It hosts a mandatory delivery records AMU database on the main livestock sectors and publishes it in the SDa report.[64] Similarly, Medirund,[65] the central database for the mandatory registration of antibiotics in cattle in the Netherlands, reports AMU data quarterly.[66]

On the medical side, the NethMap report publishes AMU data from electronic antibiotic prescriptions on patient level.[26] These are extracted from the Dutch hospital electronic prescribing system for hospitals by SWAB and from the Foundation for Pharmaceutical Statistics (SFK) system for the community. This report also assimilates data from the national sentinel surveillance network for infectious diseases in nursing homes (SNIV).[67]

United Kingdom

The VMD collates and analyses overall sales data from marketing authorization holders and aggregated usage data

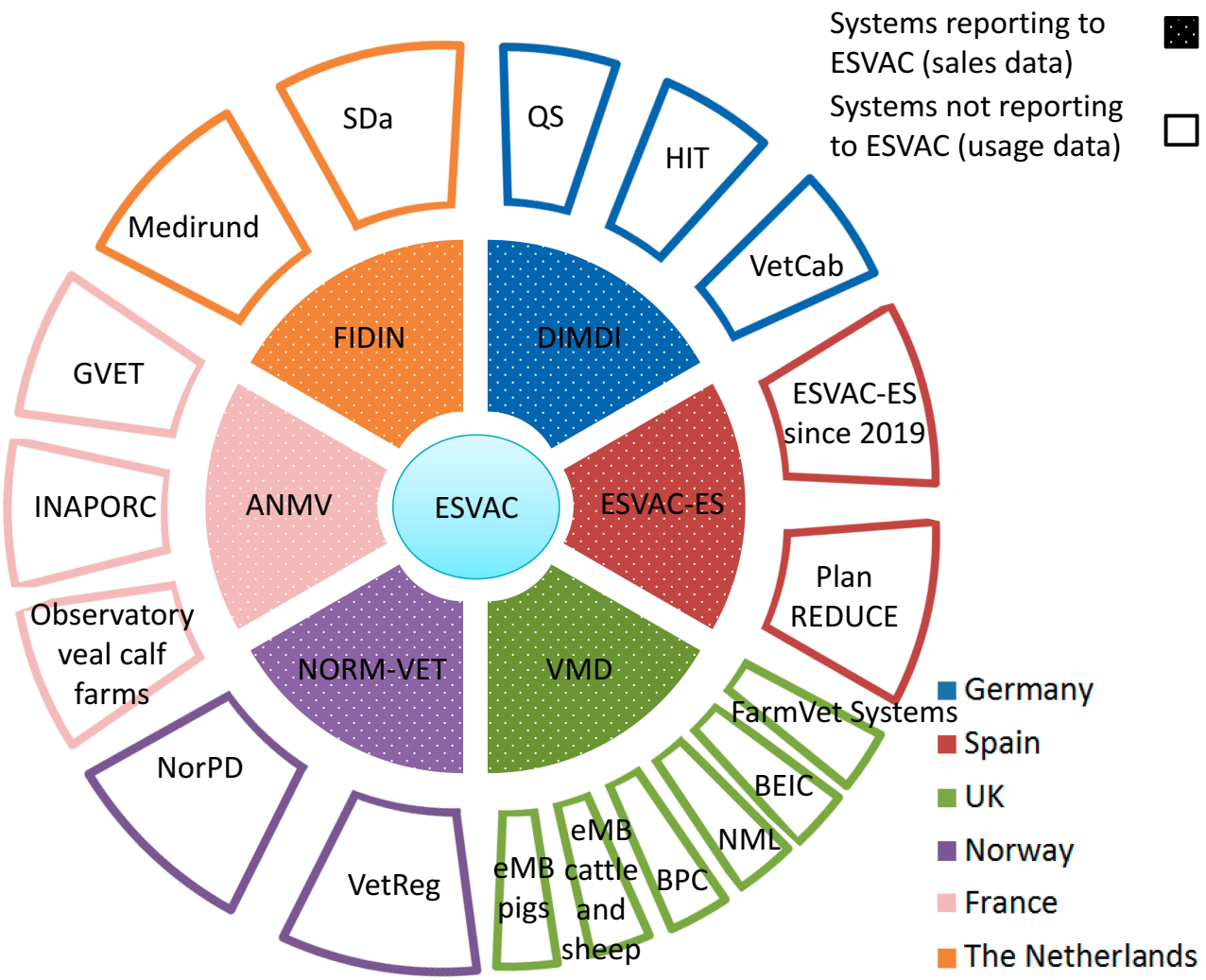

Figure 4 Overview on AMU systems in livestock in six European countries. Inner ring systems (dotted sections) report AMU data to ESVAC while outer ring systems not. For details on the systems and their relationship, see the body of the text.

by species provided on a voluntary basis by several industry-based databases. Both sales and usage data are published in the UK-VARSS report. The industry-based databases on pig, cattle and poultry are

- The Electronic Medicine Book for Pigs (eMB pigs),[68] launched by the Agriculture and Horticulture Development Board Pork (AHDB-Pork), collects usage data at farm level from the pig industry in the UK, covering around 90% of production.
- The British Poultry Council (BPC) Stewardship[69] provides meat poultry usage data (for chickens, turkeys and ducks) in the UK, covering 90% of UK poultry meat production.
- The British Egg Industry Council (BEIC) organizes the collection of antibiotic usage data for the laying hen industry. The Lion Scheme, representing over 90% of the UK laying hen industry, requires sharing usage data with BEIC.
- FarmVet Systems is a private company, which collects usage data from veterinary practice and this data is published for cattle (dairy, around 30% UK coverage, and beef, around 5% UK coverage). This represents a convenience sample and so may not be representative of the UK cattle industry.[32]

Additionally, the National Milk Laboratories (NML) database collects AMU data at farm level in dairy cattle.[70] However, this data source is currently at an early stage. Likewise, the new eMB cattle and sheep database[71] has been set up during 2018 as a pilot project collecting usage data at farm level and it is still in pilot stage.

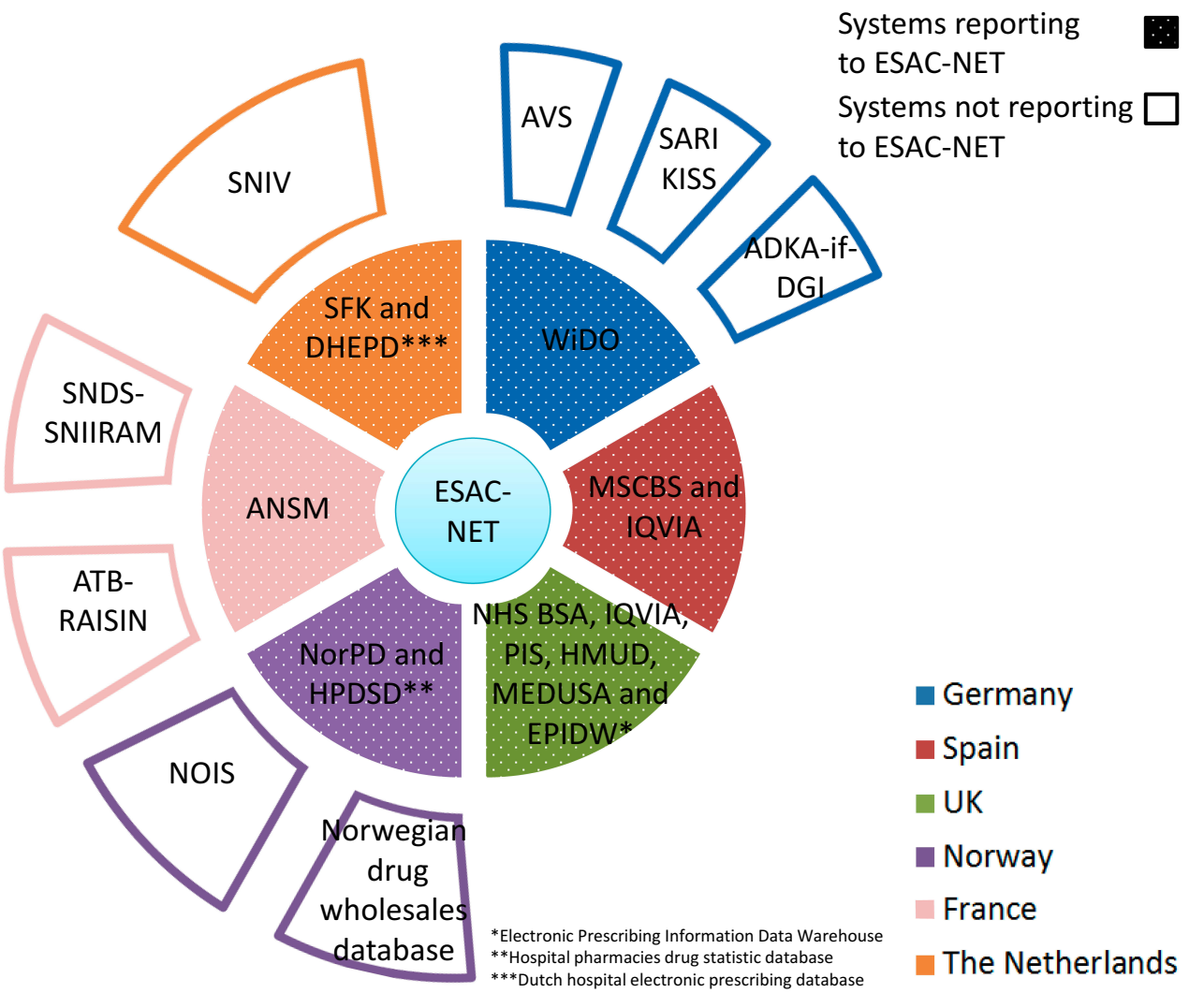

Figure 5 Overview on AMU systems in humans in six European countries. Inner ring systems (dotted sections) report AMU data to ESAC-NET while outer ring systems not. For details on the systems and their relationship, see the main text.

On the medical side, the NHS Digital database in England displays antibiotic prescribing and AMR indicators from general practice (GP). Additional primary care sources such as hospices, nursing homes, police custody (among others) are displayed in the ESPAUR report. The IQVIA database shares secondary care data with Public Health England (PHE). However, these data are not openly available.

In Scotland, the Information Service Division (ISD) holds the Prescribing Information System (PIS) database that provides AMU data from primary care. The data are supplied by the Practitioner and Counter Fraud Services (P&CFS) of the National System Scotland (NSS). This system is responsible for the processing and pricing of all prescriptions dispensed in Scotland. Data from secondary care are provided by the Hospital Medicines Utilisation Database (HMUD) that is also held by the ISD. Primary and secondary care data are published in the SONAAR report.

In Northern Ireland, the Electronic Prescribing Database and the JAC Medicines Management Systems are the two datasets on AMU data from primary and secondary care, respectively. Data are published in the NI report.

In Wales, the AMU data are collected from the prescribing Information Data Warehouse for primary care provided by the Prescribing Services Unit (PSU)[72] and the Medusa dataset[73] for secondary care. Both are managed by Public Health Wales (PHW) and they cover 100% of Welsh dispensing contractors and hospital pharmacies.

Norway

The NORM-VET monitoring system collects AMU data from the Norwegian drug wholesales statistics database

and the Veterinary Prescription Register (VetReg).[40,74] The latter register is owned by the Norwegian Food Safety Authority (NFSA) and applies to veterinarians, pharmacies and feed mills. There, prescription data on farmed fish (since 2011) and terrestrial animals (since 2012) are stored.

On the medical side, the NORM surveillance program gathers AMU data in humans from the following surveillance systems:

- The Hospital Pharmacies Drug Statistics Database.
- The national prescription database (NorPD)[75] that contains dispensed drugs in hospitals and nursing homes from pharmacies in Norway.
- The Norwegian drug wholesales statistics database that contains all sales data in Norway provided by the Norwegian Institute of Public Health (NIPH).

Additionally, the Norwegian Surveillance System for Antibiotic Consumption and Healthcare-Associated Infections (NOIS) is a nationwide mandatory system administrated by the NIPH. It is largely based on hospital automated data extraction on AMU.

France

The French Agency for Veterinary Medicinal Products (ANMV)[76] within ANSES collects annually sales data on veterinary drugs containing antibiotics in France, and also reports these data stratified by animal species. ANMV uses marketing authorisation holder estimates on the proportion sold per target species. In addition, The Permanent Observatory of Antibiotics in Veal Calf Farms[77] is a voluntary system collecting AMU data in veal calves. Data are collected and analyzed by ANSES and the French Livestock Institute (IDELE).

The French Pork Interprofessional Organization (INAPORC)[78] is a voluntary system gathering consumption data on pig categories (sows, fatteners, weaners and sucklers) from 160 randomly chosen pig farms (approximately 1% of French farms). This system is run by ANSES, the swine industry's French technical institute (IFIP) and stakeholders. The results are delivered to each farmer by the end of the study. Similar to INAPORC, the GVET[79] system is a voluntary pig register run by ANSES and IFIP. The latter system also collects AMU data from the same pig categories adopted by INAPORC providing an online result access to farmers.

On the medical side, the national health insurance cross-schemes information system (SNIIRAM) is a large French healthcare database which covers around 99% of the French population. SNIIRAM was extended with outpatient data through the National health data system (SNDS) by French law[80] in 2016. The SNIIRAM-SNDS dataset provides prescription data covering primary and secondary care in the ambulatory and hospital sectors. It includes systems such as CNAMTS (for employed workers), RSI (for independent workers) and MSA (for farmers) among others. Through CNAMTS, the MEDIC'AM spreadsheet[81,82] provides all medication reimbursement data and also the costs to the system (overall and reimbursement) and packages sold. SPF will take over shortly SNIIRAM-SNDS (2019).

In parallel, The Antibiotic Consumption Monitoring RAISIN module (ATB RAISIN) provides AMU data collected on a voluntary basis from the hospital sector. It is connected to the CPIAS network. It will be replaced shortly by the tool ConsoRes (2019).[46]

The French National Agency for Medicine and Health Products Safety (ANSM) publishes on a regular basis the antibiotic consumption trends in France report.[83] It includes data on outpatient and hospital AMU in humans, critical antibiotics and AMU in Europe. The data in the report are extracted from the following databases: ANSM, IQVIA, Permanent Sample of Medical Prescription (EPPM), OPEN-MEDIC and ESAC-Net.

Germany

Several initiatives collect AMU data in Germany. The animal antimicrobial sales data are reported annually by the industry and wholesalers to the German Institute for Medical Documentation and Information (DIMDI).[84]

The industry-based system run by Quality and Safety GmbH (QS)[85] carries out an antibiotic monitoring program on AMU data in broilers, turkeys, ducks, veal and pork productions. Some QS data are transferred to the Hi-Tier (HIT)[27] database. The HIT database is hosted by the Bavarian Ministry for the Food Chain, Agriculture and Forestry. The data collection includes treatment data on pigs, turkeys, broilers and cattle. This AMU database receives data from farmers and vets including the antimicrobial product, treatment days and number of treated animals. From the data, benchmarks for AMU in the different livestock sectors are calculated twice a year and published by the BVL. These inform farmers on the necessity to reduce AMU, ie farmers with a use above the third quartile of all reporting farms of their sector need to take action.

The sentinel project Veterinary Consumption of Antibiotics (VetCab)[27,86] is carried out by the Institute for Biometry, Epidemiology and Information Processing of the Hanover University of Veterinary Medicine Foundation (IBEI-TIHO). VetCab aims to describe and assess AMU in farm animals in Germany and includes data on pigs, cattle and broilers.

In the human sector, two national surveillance systems are in place for hospital data: AVS (RKI) and ADKA-if-DGI.

The Federal Association of German Hospital Pharmacists (ADKA) created together with the infectious disease department of the University Freiburg the ADKA-if project in 2007. Since 2015, the network supported by the German Society for Infectious Diseases (DGI) is called ADKA-if-DGI.[87]

The AVS[87,88] housed by the RKI with technical support of the Charité collects data from German and Austrian hospitals on antimicrobial consumption for individual substances and groups of substances in acute care hospitals and rehabilitation centers since 2015 (2014 pilot study) according to the German Infection Protection Act.

On behalf of the SHI (Statutory Health Insurance Funds), the WIdO[55,89] collects all antimicrobial prescriptions from mandatory health-insured patients (totaling about 89% of the German population). Only reimbursement data from the ambulatory sector (about 85–90%) are included in the WIdO dataset. Since 2001, all prescription data have become available. WIdO data are yearly reported to ESAC-Net via the RKI. In addition, the ZI, a research institute of the Federal Association of Statutory Health Insurance Physicians collects AMU data of ambulant patients.

Discussion

Harmonization and Interpretation of the AMU and AMR Data

A wide variety of AMU and AMR monitoring and surveillance systems and reports were identified at country and regional levels in the six countries. Funding of the systems is mostly public, but may also be private.

Monitoring and surveillance databases are mostly not freely accessible. Some databases do not report to the public on a regular basis. Even when most of the reports like NORM/NORM-VET, UK-VARSS, ESPAUR, MARAN/NETHMAP, ARS, EARS-Net among others, which publish aggregated data, are freely accessible others like Medirund or EASSA reports are not. This lack of free access to the available information may contribute to the existence of overlap between systems, reports and databases that may duplicate efforts and economic resources.

A further potential overlap source may be that the development of the different systems is frequently due to specific interests that are not fully covered by earlier systems leading to a substantial diversity in objectives and procedures. Therefore, it seems essential to reduce the number of overlapping systems joining forces, promoting synergies and planning the systems properly. Note that some overlap between systems may also contribute to validate system results. Some examples of overlapping are presented in Table 3. In addition, often newer systems are easier to use with the possibility of web-based reporting and feedback.

The presence of overlap between systems also translates to the existence of different reports that provide information on the same type of data generated in different systems. Furthermore, reports not based on a specific system may also produce overlaps (eg GERMAP resistance data on animals are also published by GERM-VET). However, the level of reported information may be different.

National AMU and AMR reports are published in different languages (see Tables 1 and 2). Annual publication of these reports in an international agreed language would facilitate access to published data.

AMR Surveillance and Monitoring Systems

AMR surveillance and monitoring systems vary substantially between sectors and across the countries in the type of data collected and reported. Besides the human, animal or food population studied, main sources of variability include the type of samples collected (clinical vs non-clinical samples) and the sample collection basis (voluntary, sentinel or mandatory). Both define the bacterial population that the isolates may be representative for and influence the degree of representativeness of the data.

Diversity was also observed regarding the laboratory methods (eg micro broth dilution, disk diffusion or other, automatic systems) and the reported result type (minimum inhibition concentration (MIC), inhibition zone (IZ) or susceptible-intermediate-resistant (SIR)). The laboratory method selected may affect final results. As an example, colistin, a key antimicrobial in human and animal health, diffuses poorly into the agar medium. Therefore, disk diffusion results from colistin are not reliable.[90] Quantitative data allow for interpretation using different

Table 3 Complementary Systems with Some Overlap

	On the Human AMR Sector	On the Human AMU Sector	On Livestock AMU Sector	On the Livestock AMR Sector	On the Food AMR Sector
Germany	ARS, PEG, MRSA-KISS, ICU-KISS, OP-KISS and SARI-KISS Regional: ARMIN and BARDa	AVS, ADKA-if-DGI SARI-KISS	DIMDI, HIT, QS and VetCab	x	x
The Netherlands	x	x	FIDIN, SDa and MEDIRUND	x	x
Norway	x	The Norwegian drug wholesales statistics database, NorPD, NOIS and the hospital pharmacies drug statistics database	NorPD, NORM VET and VetReg	x	x
United Kingdom	BSAC Regional: SGSS, ECOSS, Datastore and CoSurv	x	FarmVet Systems, eMB cattle and NML	x	x
France	BMR-RAISIN and ONERBA	SNDS-SNIIRAM, ANSM, ATB-RAISIN	ANMV, GVET and INAPORC	x	x
Spain	x	MSCBS and IQVIA	ESVAC-ES and Plan REDUCE	x	x
Europe	x	x	x	x	EASSA and EFSA

clinical breakpoints (CBP) or epidemiological cut-offs (ECOFF) as provided by EUCAST, CLSI or other, sometimes national institutions. SIR data can only be validly compared to other SIR data, if the methodology used is standardized. This includes both laboratory methods as well as the ECOFFS or breakpoints used for the categorization of the isolate populations to SR or SIR. Therefore, AMR reports should capture quantitative data rather than qualitative values (SIR or SR) to allow for interpretation of data using different thresholds. However, the comparability of quantitative data from different laboratory methodologies remains as an issue.

Data collection systems often adopt a specific standard. However, most standards and their corresponding evaluation criteria do not cover all drug/bug combinations. In that case, different standards and/or evaluation criteria may be used for different drug/bug combinations in the same data collection system. Therefore, AMR collecting systems should have a similar approach (ie standard, evaluation criteria, antibiotic panel, unit and data type (clinical-non clinical)) so that data comparison, evaluation and analyses across countries and sectors were valid.

In addition, ECOFFs and CBPs are regularly revised, so their threshold values may vary over time. Differences between ECOFFs and CBPs are frequently underlined in literature.[91,92] ECOFFs identify the wild-type (those assumed to have no acquired/mutational resistance) from non-wild-type populations (those that show a degree of acquired/mutational resistance) while CBPs define clinically a microorganism as "sensitive", "intermediate" or "resistant" in relation to the likelihood of therapeutic success. CBPs take into account information such as the infection site, ability of the antimicrobial to reach the infection site, dosage regimens and formulations available to determine the effectiveness against the pathogen. Therefore, interpretation of results between countries may not be directly comparable as different dose regimens are used.[93] However, in most instances, the differences between published ECOFF and CBP values are limited, given that one dilution step is the tolerance of microdilution in both systems. Moreover, values for both evaluation criteria are constantly evolving when new data become available and those with the greatest differences (eg ciprofloxacin in *E. coli*) tended to converge over time.[94]

The main task of surveillance systems is to provide an overview of patterns and trends, however some systems may provide additional useful information for risk factor analysis.

A further source of variability identified on AMR systems is the collection of sample results from diverse

laboratories, using different diagnostic methods and interpretation standards. Antimicrobial panels to be tested in laboratories against zoonotic and commensal bacteria are standardized in the livestock sector by Commission Implementing Decision 2013/652/EU. This is not the case in the medical sector and for the testing of clinical isolates from animals. However, EU institutions clearly indicate that the antimicrobial panel described for indicator bacteria on livestock by Decision 2013/652/EU takes human relevance into consideration.[95] Therefore, reporting part of the data adopting this standardized panel would help minimize current standardization and harmonization issues.

AMU Surveillance and Monitoring Systems

AMU collection systems are based on a variety of data sources ranging from overall national sales data to individual prescription or treatment data.[7] Data are displayed in very diverse units (such as weight of active ingredient, therapy frequency, mg/Population Correction Unit (PCU), Defined Daily Dose (DDD)/1000 inhabitants/days, DDD/1000 Specific Therapeutic Group Age-sex weightings Related Prescribing Units (STAR-PU) among others) hampering the comparison of data from different sources.

Differences in dosage regimes and treatment durations between hospitals and countries might result in an erroneous assessment of the treatment numbers if they are deduced from the amount of drug sold. For these reasons, any evaluation and comparison of AMU data from different sources should be done carefully.

A consensus has been reached to report AMU data to European level (ESVAC and ESAC-Net systems) adopting the unit DDD/1000 inhabitants/days on the medical side and antibiotic weight per population correction unit (mg/PCU) on the animal side. However, as with breakpoints in AMR, these units have drawbacks and the consensus is a compromise that is continuously under debate.

The weight of the active ingredient as collected for the ESVAC project does not account for its potency, ie the amount needed to treat 1 kg of animal. Moreover, most antimicrobials may be used in several animal species and may also be licensed at a different dosage for different and sometimes even for the same animal species. Sales data on the veterinary side therefore only provide a general overview, but for further analyses, farm-level data are needed. These are frequently collected on the regional or national level as shown in Table 2, but at a very low level of harmonization. At best they allow for assessing trends within the system, but between systems analyses are very challenging. This also applies for comparisons to the medical side. The differences between the systems have repeatedly been described and critically reviewed.[27,96]

The DDD/1000 inhabitants/day is widely used as a standard for monitoring antimicrobial consumption for the human sector. However, it does not necessarily reflect the dose prescribed to the individual patient. This particularly plays a role for special patient populations (eg children or patients with renal insufficiency). The same issue applies for defined daily doses for animals. Dosing of drugs for systemic use ideally should be done giving the amount of drug needed per kg of treated individual. If the weight of the treated animal is not accounted for and DDDs are calculated from the amount of drug used alone, substantial miscalculations are possible. In broilers 1-day-old chicks weigh about 50 g and 1-month-old broilers around 2 kg, ie, that is 40 times more. If 1 kg of drug dosed at 20mg/kg/day can be used to treat 1,000,000 1-day-old chicks, it will only serve 25,000 1-month-old broilers. Using a standard weight for broilers at about 1 kg (average weight at the time of treatment applied by ESVAC) to calculate a DDD would result in 50,000 DDDs which neither reflects the exposure of 1-day-old chicks nor the exposure of 1-month-old broilers.

Therapy frequency, used for farm animals in Germany, on the other hand, has the drawback that it does not account for dosing as it only considers the number of animals that were treated with the drug, assuming that this happens at a standard dose. DDDs, in case the DDD are equivalent to the prescribed daily dose (PDD), and therapy frequency both may represent days under treatment, but the results may differ substantially when describing the same population. This is because one is based on counted treatment days and therefore the amount of active substance used cannot be deduced from the figure. DDD, on the other hand, is deduced from used amounts of drugs and therefore does not have to be equivalent to real treatment days because of the issues explained above.

In summary, regarding antimicrobial use in animals, there is need for a measure that includes the name of the active ingredient, the amount of active ingredient, the number of treated animals, the population at risk, the weight of treated animals, the time under treatment and the duration of the therapeutic effect of the active ingredient in the body. If those are collected, most of the units that are currently in use should be deducible from the information

with a reasonable accuracy. Likewise, besides DDDs, additional metrics should be collected in order to describe the different aspects of AMU in the human sector (eg days of treatment, number of prescriptions).

However, the EU agreed data type is not always provided in the national or regional surveillance and monitoring system reports but other units such as DDD/1000 STAR-PU are.[97]

Those datasets that do not report their data at European level may have different units than the agreed ones, such as HIT or the SNIIRAM-SNDS system.

The health-care system implemented in each country is of great relevance to understand the data collection. As an example, in England, it is common to dispense outpatient medications by hospitals, whereas in Northern Ireland these are usually prescribed by the GP at the request of secondary care specialists.[98] Thus, there may be significant dissimilarities in the data collected across countries from homologous databases.

The usage data per animal species is a more useful source than sales data; however, it is not consistently collected by all countries and also not provided to ESVAC yet. Collections of these data are laborious if they are not available in electronic formats. However, in most countries, prescription data are collected at least from a part of the animal population such as in the UK (collecting prescription data on a voluntary basis from pigs through eMB pigs and meat poultry through BPC stewardship), among others or Norway (mandatory data collection of prescription data from food-producing animals in VetReg and on a voluntary basis from companion animals). However, the VetReg system has been compared to the sales data and there is a proportion of underreporting among the prescription data recorded since the registry started.[99]

Tools for Comparison

JIACRA analyses comparing AMU in animals and humans to AMR in the sectors are accompanied by a long list of disclaimers but provide a valuable general overview. Major progress could further be improved by including prescription data, or at least use data by animal species.

Diverse tools have been developed to assess correlations and associations between AMU and the development of AMR. As an example, the hospital-based ARVIA[100] and Conso-Res are similar initiatives under development in the human sector launched by Germany and France, respectively. These efforts supplement the JIACRA reports and address the issue at the hospital level.

Conclusions

- AMU and AMR Systems and Reports Need Further Harmonization to Support the One Health approach.
- Availability of prescription data or similar for animals would allow a more detailed analysis of antimicrobial treatment and resistance data, and enhance interpretation of the findings published in the JIACRA reports by EFSA, EMA and ECDC.
- In addition, major challenges need to be addressed in order to harmonize AMU and AMR data in the animal sector through uniform and robust standards that are either fully harmonized or allow for conversion of data to different units. To this end, for AMU, the name of the active ingredient, amount of active ingredient, number of treated animals, the population at risk, weight of treated individual, treatment duration and the duration of the therapeutic effect of the active ingredient in the body are needed.
- AMR collecting systems should have as far as possible a similar approach (ie standard, evaluation criteria, antibiotic panel, unit and data type (clinical-non clinical)) to be compared, evaluated and analyzed across countries and sectors. Otherwise, the data may not be directly assessed. Additionally, reports on AMR should capture quantitative values rather than data on the SIR level to allow for interpretation of data using different thresholds. However, there will be still an issue with comparability of quantitative data from different methodologies.
- ECDC, EMA and EFSA indicate that the antimicrobial panel described for livestock by Decision 2013/652/EU takes human relevance into consideration. Reporting at least part of the data adopting this standardized panel would ensure uniformity.
- Currently, there is some overlap between national and international systems (see Table 3). Therefore, it seems essential to join forces, promote synergies and plan the systems properly in order to avoid overlapping and address potential gaps making better use of the available resources. A first step to achieve the latter goal is to address the system harmonization that will substantially increase data sharing with the EU. It seems that some resources could be used more efficiently by reducing the number of overlapping systems. However, note that some overlap between systems may be useful for system and data validation.
- Preferably national AMU and AMR reports should be published annually and provided in one international

agreed language (eg English) to facilitate access to published data.

Abbreviations

AACTING, Network on quantification of veterinary Antimicrobial usage at herd level and Analysis, CommunicaTion and benchmarkING to improve responsible usage; ADKA, Federal Association of German Hospital Pharmacists; AECOSAN, Spanish Agency for Consumer Affairs, Food Safety and Nutrition; AEMPS, Spanish Agency of Medicaments and Sanitary Products; AHDB, Agriculture and Horticulture Development Board; AMR, Antimicrobial Resistance; AMU, Antimicrobial Use; ANMV, National Agency for Veterinary Medicines; ANSES, French Agency for Food, Environmental and Occupational Health & Safety; ANSM, French National Agency for Medicine and Health Products Safety; APHA, Animal and Plant Health Agency; ARDIG, Antibiotic Resistance Dynamics: the influence of geographic origin and management systems on resistance gene flows within humans, animals and the environment; ARMIN, Antibiotic Resistance Monitoring in Lower Saxony; ARS, Antibiotics Resistance Surveillance; AST, Antibiotic Susceptibility Testing; ATB RAISIN, Antibiotic Consumption Monitoring programme; AVS, Antibiotic Consumption Surveillance; BDCAP, Primary Care Clinical Database; BfR, Federal Institute for Risk Assessment; BEIC, British Egg Industry Council; BIFAP, Database for Pharmacoepidemiological Research in Primary Care; BPC, British Poultry Council Stewardship; BSAC, British Society for Antimicrobial Chemotherapy; BVL, Federal Office of Consumer Protection and Food Safety; CAESAR, Central Asian and Eastern European Surveillance of Antimicrobial Resistance network; CA-SFM, Antibiogram Committee of the French Society for Microbiology; CBP, Clinical Breakpoints; CCLIN, Nosocomial Infection Surveillance Coordination Centers in France; CEESA, European Animal Health Study Center; CLSI, Clinical Laboratory Standard Institute; CPIAS, National Network for the Prevention of Care-Related Infections; DARC, Defra Antibiotic Resistance Coordination; DART, German antibiotic resistance strategy; DCD, Defined Course Dose; DDD, Defined Daily Dose; DGI, German Society for Infectious Diseases; DIMDI, German Institute for Medical Documentation and Information; DIN, German Institute for Standardization; EARS-Net, European Antimicrobial Resistance Surveillance Network; EASSA, European Antimicrobial Susceptibility Surveillance in Animals; ECDC, European Center for Disease Prevention and Control; ECOFF, Epidemiological Cut-Off; EFFORT, Ecology from Farm to Fork Of microbial drug Resistance and Transmission; ECOSS, Electronic Communication of Surveillance in Scotland; EFSA, European Food Safety Authority; EJP, European Joint Programme; EMA, European Medicines Agency; eMB cattle and sheep, Cattle and sheep Electronic Medicine Book; eMB pigs, Pig Electronic Medicine Book; EPPM, Permanent Sample of Medical Prescription; ESAC-Net, European Surveillance of Antimicrobial Consumption Network; ESPAUR, English Surveillance Programme for Antimicrobial Utilisation and Resistance; ESVAC, European Surveillance of Veterinary Antimicrobial Consumption; EU, European Union; EUCAST, European Committee on Antimicrobial Susceptibility Testing; FIDIN, Federation of the Dutch veterinary pharmaceutical industry; GAP, Global Action Plan; GERMAP, Antibiotic Consumption and the Spread of Antibiotic Resistance in Human and Veterinary Medicine in Germany; GERM-VET, German Veterinary Monitoring System; GP, General Practice; HIT, German animal movement and information system; IBEI-TIHO, Institute for Biometry, Epidemiology and Information Processing of the Hanover University of Veterinary Medicine Foundation; IDELE, French Livestock Institute; IFIP, Swine Industry's French Technical Institute; INAPORC, French Pork Interprofessional Organisation; IQVIA, Human Data Science Company; ISCIII, Carlos III Health Institute; ISD, Information Service Division; ISIS-AR, Infectious Disease Surveillance Information System on Antibiotic Resistance; IZ, Inhibition Zone; JIACRA, Joint Interagency Antimicrobial Consumption and Resistance Analysis; KISS, Hospital Infection Surveillance System; MABUSE, Medical Antimicrobial Use Surveillance and Evaluation; MAPAMA, Ministry of Agriculture, Fisheries and Food; MARAN, Monitoring of Antimicrobial Resistance and Antibiotic Usage in Animals in the Netherlands; Medirund, Central Database for the Mandatory Registration of Antibiotics in Cattle in Netherlands; MIC, Minimum Inhibitory Concentration; MS, Member State; MSIS, Norwegian Surveillance System for Communicable Diseases; NethMap, Consumption of Antimicrobial Agents and Antimicrobial Resistance Among Medically Important Bacteria in the Netherlands; NFSA, Norwegian Food Safety Authority; NHS, National Health Service; NIPH, Norwegian Institute of Public Health; NOIS, Norwegian Surveillance System for Antibiotic Consumption and Healthcare-Associated

Infections; NORM, surveillance programme for antimicrobial resistance in human pathogens; NORM-VET, Monitoring Programme for Antimicrobial Resistance in the Veterinary and Food Production sectors; NorPD, Norwegian Prescription Database; NSS, National System Scotland; NVI, Norwegian Veterinary Institute; NVMM, Ministry of Health, Welfare and Sport and the Dutch Society of Medical Microbiology; NVWA, Netherlands Food and Consumer Product Safety Authority; ONERBA, National Observatory of the Epidemiology of Bacterial Antibiotic Resistance; P&CFS, Practitioner and Counter Fraud Services; PEG, Paul Ehrlich Society for Chemotherapy; PHE, Public Health England; PHW, Public Health Wales; PIS, Prescribing Information System; PRAN, National Antibiotic Resistance Plan; PSU, Prescribing Services Unit; QS, Quality and Safety GmbH; RAISIN, Alert, Investigation and Surveillance of Nosocomial Infection Network; RDD, Recommended Daily Dose; RESAPATH, French Surveillance Network for Antimicrobial Resistance in Pathogenic Bacteria of Animal Origin; RIVM, National Institute for Public Health and the Environment; RKI, Robert Koch Institute; SARI, Surveillance of Antimicrobial Use and Bacterial Resistance in Intensive Care Units; SPF, French Health System; SDa, Netherlands Veterinary Medicines Institute; SGSS, Second Generation Surveillance System; SHI, Statutory Health Insurance; SIR, Sensible, Intermediate, Resistant; SNDS, French National Health Data System; SNIIRAM, French National Health Data System; SNIV, National sentinel surveillance network for infectious diseases in nursing homes; SONAAR, Scottish One Health Antimicrobial Use and Antimicrobial Resistance; SRUC, Scotland's Rural College Veterinary Services and Capital Diagnostics; STAR-PU, Specific Therapeutic Group Age-sex weightings Related Prescribing Units; SWAB, Dutch Foundation of the Working Party on Antibiotic Policy; UK, United Kingdom; UK-VARSS, UK-Veterinary Antibiotic Resistance and Sales Surveillance; VAV, Spanish Veterinary Antimicrobial Resistance Surveillance Network; VetCab, Sentinel project Veterinary Consumption of Antibiotics; VetReg, Veterinary Prescription Register; VMD, Veterinary Medicines Directorate; WHO, World Health Organization; WIdO, Scientific Institute of the AOK; ZOMO, German Zoonosis Monitoring.

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Chapter 4: Phenotypical antimicrobial resistance data of clinical and non-clinical *Escherichia coli* from poultry in Germany between 2014 and 2017.

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RESEARCH ARTICLE

Phenotypical antimicrobial resistance data of clinical and non-clinical *Escherichia coli* from poultry in Germany between 2014 and 2017

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Abstract

Antimicrobial resistance (AMR) is a global threat in humans and animals, and antimicrobial usage (AMU) has been identified as a main trigger of AMR. The purpose of this work was to compare data on AMR in clinical and non-clinical isolates of *Escherichia coli* in German broilers and turkeys between 2014 and 2017. Furthermore, we investigated AMR changes over time and the association of changes in AMU with changes in AMR. Data on clinical and non-clinical isolates together with data on therapy frequency of broilers and turkeys were collected from German monitoring systems. Logistic regression analyses were performed to assess the association between the explanatory factors (AMU, year and isolate type) and the dependent variable (AMR). In broilers, the analysis showed lower resistance proportions of clinical isolates of *E. coli* to ampicillin and colistin (ampicillin: Odds ratio (OR) and 95% confidence interval (CI) = 0.44 (0.3–0.64), $p<0.001$; colistin: OR and 95% CI = 0.75 (0.73–0.76), $p<0.001$) but higher proportions for cefotaxime (OR and 95% CI = 4.58 (1.56–15.1), $p = 0.007$). Resistance to ampicillin, gentamicin and tetracycline was less frequent in clinical isolates in turkeys (ampicillin: OR and 95% CI = 0.4 (0.29–0.53), $p<0.001$; gentamicin: OR and 95% CI = 0.5 (0.26–0.94), $p = 0.035$; tetracycline: OR and 95% CI = 0.4 (0.29–0.55), $p<0.001$). The analysis found decreasing associations of AMU with resistance to tetracycline in turkeys and to colistin in broilers. Year was associated with a decrease in resistance to colistin in broilers and to tetracycline in turkeys. Differences in resistance found in this study between clinical and non-clinical isolates might play an important role in resistance prevalence. This study indicated that further data analyses over longer time intervals are required to clarify the differences found between clinical and non-clinical isolates and to assess the long-term effects of changes in AMU on the prevalence of AMR.

Introduction

Antimicrobial resistance (AMR) is a global threat that has increased in recent years in humans and animals. Antimicrobial usage (AMU) has been identified as a main trigger of AMR [1, 2].

European Joint Programme (EJP) on AMU and AMR in humans, food and animals. ARDIG project has received funding from the European Union's Horizon 2020 research and innovation programme under Grant Agreement No 773830. URL: https://ec.europa.eu/programmes/horizon2020/en. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

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An increase of AMU is expected in most underdeveloped countries in the coming years [3]. Large differences in AMU [4] and AMR [5–8] shown across the countries such as Spain, Italy, Norway or Sweden evidence clearly this relationship.

Global strategies have been developed to tackle this threat such as the Global Action Plan (GAP) of the World Health Organization (WHO) [9] or the new European One Health Action Plan against AMR [10]. The "Deutsche Antibiotika-Resistenzstrategie" (DART), is the national action plan (NAP) in Germany. It was first set up in 2008 in line with the recommendations made at the European level.

As a part of NAPs, surveillance and monitoring systems are essential to gather crucial information such as prevalence, incidence, trends, resistance patterns and key drivers of resistance. The systems may help to improve the global understanding of AMR helping decision makers to take appropriate actions to minimise or even prevent the spread of AMR [11]. NAPs also promote many governmental initiatives and projects that collect valuable information addressing new prevention strategies.

In relation to the collection of AMR data, historically two types of bacterial populations have been established: (a) The population collected from animals without underlying pathologies (non-clinical data; i.e. commensals) and (b) the population from diseased animals (clinical data).

In Europe, the majority of AMR data on non-clinical isolates from livestock come from standardized monitoring systems based on Commission Implementing Decision 2013/652/EU. Data are collected by the European Food Safety Authority (EFSA) [12]. On the other hand, the VetPath monitoring system, an initiative funded by the pharmaceutical industry, collects data on clinical isolates in livestock at European level [11, 13, 14]. However, the number of isolates is limited and data is not freely available. Some European countries have additionally set up programmes collecting data on clinical isolates from animals (e.g. France, Norway, United Kingdom and Germany) [11].

In Germany, in recent decades, poultry and particularly the broiler meat sector has increased its relevance as a meat source. In 2019, poultry production reached 1,918,000 tons carcass weight, of which 1,340,000 were from broilers [15].

While Europe banned antimicrobial grow promoters in 2006 [16], antimicrobials are still widely used in the poultry sector [17–19]. They are prescribed/administered to the flocks as a therapy against diseases or during metaphylactic treatment. Antimicrobials approved for use in poultry in Germany are neomycin, spectinomycin, amoxicillin, ampicillin, benzylpenicillin, phenoxymethyl-penicillin, trimethoprim, lincomycin, tylosin, tilmicosin, tylvalosin, tiamulin, colistin, enrofloxacin, sulfadimethoxine, sulfadimidine, sulfamethoxazole, sulfaquinoxaline, doxycycline and oxytetracycline [20].

Escherichia coli are Gram-negative bacteria commonly found in the intestine of animals as commensal microorganisms. They are also a main threat for the poultry sector causing animal disease and considerable economic losses [20]. *Escherichia coli* may serve as a reservoir spreading resistance genes horizontally to other bacteria [21]. The emergence of AMR due to AMU can be evaluated through the monitoring of resistant *E. coli*, a widely accepted AMR indicator [8, 22–24]. The relation between AMU and AMR has been extensively described in livestock in general [25], in pigs [26–28], in cattle [28–30], and in poultry [28, 31–34]. A large number of publications evaluate the *E. coli* resistance proportions in poultry without analytically considering AMU [35–42].

In 2019, a study carried out in Estonia collected AMR data on clinical and non-clinical isolates in pigs and cattle. In this study, higher proportions of resistance were observed in clinical isolates than in non-clinical isolates on the descriptive level [43], but no statistical analysis comparing both isolate types was carried out. To our knowledge, there are no publications comparing data on AMR in clinical and non-clinical isolates from poultry.

The main objective of this work is therefore to compare data on AMR in clinical and non-clinical isolates of *E. coli* from German broilers and turkeys. It would be reasonable to expect the level of resistance to be higher in clinical isolates compared to non-clinical isolates, as diseased broilers and turkeys may carry bacteria resistant to regular antimicrobial treatments [43]. Furthermore, we investigate AMR changes over time and the association of changes in AMU with changes in AMR. We challenge two hypotheses in this manuscript: (1) The level of AMR in *E. coli* from broilers and turkeys is higher in clinical isolates than in non-clinical isolates and (2) there is a demonstrable association between changes in AMU and changes in AMR in isolates from broilers and turkeys. In order to challenge our two hypotheses, we applied univariate and multivariate logistic regression analyses comparing resistance prevalence of clinical and non-clinical isolates of *E. coli* from broilers and turkeys. Further variables also included in the analyses were: (1) year (2014 to 2017) and (2) AMU (in broilers and turkeys).

Materials and methods

Data collection and processing

Phenotypic resistance data on clinical and non-clinical isolates of *E. coli* were collected from two different sources from 2014 to 2017. Data on non-clinical isolates from caecal samples originated from the German Zoonosis-Monitoring programme (ZoMo) [44]. Data on clinical isolates from different sample types originated from the German Resistance Monitoring of Veterinary Pathogens (GE*RM*-VET) [45]. Data on antimicrobial susceptibility testing (AST) of clinical and non-clinical *E. coli* isolates had both been obtained by broth microdilution [44, 45].

Duplicate isolates were eliminated prior to data collection preventing bias during the analysis process. To avoid a major influence of individual isolates, data were only included in the analysis when more than 24 isolates were tested and reported per year, category (clinical/non-clinical), antimicrobial drug / antimicrobial class and animal species. The antimicrobial panel analysed included cefotaxime, ciprofloxacin, colistin, nalidixic acid, tetracycline, gentamicin and ampicillin. This panel reflected the overlap between the test panels used in the two monitoring programs for clinical and non-clinical isolates.

German AMU data for broilers and turkeys were available as total amount of active ingredient in tons and as therapy frequency (TF) [17]. Therapy frequency was selected in this manuscript as a more accurate AMU parameter expressing animal exposure in days under treatment and it was used to study the association of AMU with AMR in the animal populations. Therapy frequency had been calculated using the following formula:

$$TF = (N^{\circ}At \times N^{\circ}TD \times N^{\circ}AI) \div N^{\circ}As \quad (1)$$

Where "N˚At" referred to the number of animals treated, "N˚TD" to the number of treatment days, "N˚AI" to the number of active antimicrobial substances and "N˚As" to the average number of animals in 6 months [17]. Therapy frequency values were available per semester in the database. Data for the first semester of 2014 were not available as the obligation to record the treatments in a central database started in July 2014. Therefore, second semester data of 2014 were doubled to obtain a TF approximation of the year (Table 1). Each drug belonging to the resistance panel was compared to the TF of its antimicrobial class (Table 2).

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was determined by broth microdilution according to CLSI standard [46]. Minimum Inhibitory Concentrations (MIC) were interpreted according to Epidemiological Cut-off values (ECOFFs) provided by EUCAST (01. September 2019) (Table 2).

Table 1. Therapy frequency, an AMU unit applied in Germany, with antimicrobial classes of broilers and turkeys from 2014 to 2017 [17].

Animal species	Antimicrobial class	Therapy frequency per year			
		2014	2015	2016	2017
Broiler	Aminoglycosides	11.66	7.68	8.56	11.32
	Cephalosporins	0.0	0.0	0.0	0.0
	Penicillins	8.76	6.25	5.77	5.54
	Polymyxins	6.88	5.56	5.03	5.73
	Fluoroquinolones	3.52	3.68	2.99	3.17
	Tetracyclines	0.82	0.33	0.35	0.44
Turkey	Aminoglycosides	1.18	1.22	1.22	1.16
	Cephalosporins	0.0	0.0	0.0	0.0
	Penicillins	31.22	26.9	23.18	25.55
	Polymyxins	12.24	9.34	7.99	7.84
	Fluoroquinolones	6.94	6.24	5.11	5.08
	Tetracyclines	4.54	3.83	3.24	2.77

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Isolates with a MIC up to the ECOFF, i.e. wild-type isolates (isolates without acquired/mutational resistance [8]), were considered susceptible while isolates with a MIC above the ECOFF, i.e. non-wild type isolates (isolates with acquired/mutational resistance [8]), resistant.

Statistical analysis

Data were managed and analysed using "the Konstanz information Miner (KNIME)" tool (Version 3.7.2) and the software "R" (Version 3.4.3) using the CRAN Packages "pscl", "logistf" and "ROCR". Data were analysed by univariate and multivariate logistic regression models adopting a binomial distribution. The dependent variable was the MIC categorization by EUCAST ECOFFs (i.e. resistant (y = 1) or susceptible (y = 0)). (i) Isolate type (clinical vs. non-clinical isolates), (ii) year and (iii) TF per antimicrobial class were included as explanatory factors for each antimicrobial of the AMR panel and animal species (broilers and turkeys). A univariate analysis was performed for each animal species and antimicrobial, assessing the association of each explanatory variable with the dependent variable. In the case of the (fluoro-) quinolones, similar univariate analyses were carried out for antimicrobial class duplicating isolates (i.e. a value for each drug) and each animal species in order to assess the relationship between each explanatory factor and the outcome variable for the entire antimicrobial class.

Multivariate analysis was carried out only when more than one variable per antimicrobial/antimicrobial class and animal species in the univariate analysis showed an association to the outcome variable with a *p*-value lower than 0.1. The level of significance for the univariate and

Table 2. Antimicrobial classes, antimicrobial agent/substance tested and epidemiological cut-offs applied to categorize antimicrobial susceptibility testing results from broth microdilution based on EUCAST (01. September 2019).

Antimicrobial class	Antimicrobial agent/substance tested	Epidemiological cut-off values (mg/L) defining the non-wild type
Penicillins	Ampicillin	>8
Polymyxins	Colistin	>2
(fluoro-)quinolones	Ciprofloxacin	>0.064
	Nalidixic acid	>16
Tetracyclines	Tetracycline	>8
Aminoglycosides	Gentamicin	>2
Cephalosporins	Cefotaxime	>0.25

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multivariate analysis was a *p*-value lower than 0.05. A multivariate analysis performed in broilers for colistin showed a complete or quasi-complete separation in the logistic regression [47] providing overestimated coefficients. In this case, the outcome variable separated the combination of predictor variables. A valid method penalising the likelihood was performed to overcome this issue in this analysis [48]. For the explanatory variable "Isolate type", an odds ratio (OR) <1 indicated a lower fraction of resistance in the clinical isolates compared to non-clinical isolates. An OR >1 indicated a higher fraction of resistance in the clinical isolates compared to the non-clinical isolates. The year and the TF, in the model, were analysed as numeric variables. *p*-values were obtained by the use of Wald Chi-square test. A *p*-value of less than 0.05 was considered statistically significant.

Results

Resistance percentages, number of resistant isolates and total number of isolates tested per year and antimicrobial in broilers and in turkeys are summarized in Tables 3 and 4 respectively. Results of the univariate and multivariate logistic regression models are shown in Tables 5 and 6.

Broilers

A total of 185 clinical isolates and 407 non-clinical isolates were collected from broilers between 2014 and 2017. In 2014, less than 25 clinical isolates were submitted and reported and were therefore excluded from the analysis. The highest resistance proportions in non-clinical isolates (>50.0%) were observed to ampicillin (2014 and 2016), nalidixic acid (2016) and ciprofloxacin (2016). In clinical isolates, highest resistance prevalence was found to ampicillin (2017), ciprofloxacin (from 2015 to 2017) and nalidixic acid (from 2015 to 2016). High levels of resistance (30.0% < 50.0%) were also found to ampicillin (from 2015 to 2016), nalidixic acid

Table 3. Number and proportion of resistant isolates of the tested clinical and non-clinical isolates of *Escherichia coli* reported from broilers in Germany 2014–2017.

Drug / drugs (class)	Type of isolate	N° of resistant / N° of tested (% resistant)			
		2014	2015	2016	2017
Ampicillin (penicillins)	Clinical	9/18 (50.0%) [a]	23/76 (30.3%)	16/50 (32.0%)	23/41 (56.1%)
	Non-clinical	128/230 (55.7%)		105/177 (59.3%)	
Cefotaxime (cephalosporins)	Clinical	0/18 (0.0%) [a]	3/76 (3.9%)	4/50 (8.0%)	2/41 (4.9%)
	Non-clinical	3/230 (1.3%)		1/177 (1.1%)	
Ciprofloxacin (fluoroquinolones)	Clinical	3/6 (50.0%) [a]	50/75 (66.7%)	31/50 (62.0%)	22/41 (53.7%)
	Non-clinical	110 (47.8)		106/177 (59.9%)	
Ciprofloxacin and nalidixic acid ((fluoro-)quinolones) [b]	Clinical	11/24 (45.8%) [a]	101/151 (66.9%)	60/100 (60.0%)	41/82 (50.0%)
	Non-clinical	213/460 (46.3%)		206/354 (58.2%)	
Colistin (polymyxins)	Clinical	1/18 (5.6%) [a]	1/76 (1.3%)	0/50 (0.0%)	0/41 (0.0%)
	Non-clinical	16/230 (7.0%)		7/177 (4.0%)	
Gentamicin (aminoglycosides)	Clinical	1/18 (5.6%) [a]	2/75 (2.7%)	6/50 (12.0%)	3/41 (7.3%)
	Non-clinical	16/230 (7.0%)		12/177 (6.8%)	
Nalidixic acid (quinolones)	Clinical	8/18 (44.4%) [a]	51/76 (67.1%)	29/50 (58.0%)	19/41 (46.3%)
	Non-clinical	103/230 (44.8%)		100/177 (56.5%)	
Tetracycline (tetracyclines)	Clinical	8/18 (44.4%) [a]	13/75 (17.3%)	7/50 (14.0%)	13/41 (31.7%)
	Non-clinical	77/230 (33.5%)		49/177 (27.7%)	

[a] not included in the analysis as less than 25 isolates were tested.

[b] considering resistance to ciprofloxacin and nalidixic acid.

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Table 4. Number and proportion of resistant isolates of the tested clinical and non-clinical isolates of *Escherichia coli* reported from turkeys in Germany 2014–2017.

Drug / drugs (class)	Type of isolate	N° of resistant / N° of tested (% resistant)			
		2014	2015	2016	2017
Ampicillin (penicillins)	Clinical	31/82 (37.8%)	38/104 (36.5%)	36/95 (37.9%)	36/63 (57.1%)
	Non-clinical	118/184 (64.1%)		119/188 (63.3%)	
Cefotaxime (cephalosporins)	Clinical	0/82 (0.0%)	0/104 (0.0%)	2/95 (2.1%)	0/93 (0.0%)
	Non-clinical	4/184 (2.2%)		4/188 (2.1%)	
Ciprofloxacin (fluoroquinolones)	Clinical	19/45 (42.2%)	31/104 (29.8%)	29/95 (30.5%)	19/63 (30.2%)
	Non-clinical	75/184 (40.8%)		61/188 (32.4%)	
Ciprofloxacin and nalidixic acid ((fluoro-)quinolones) [a]	Clinical	59/126 (46.8%)	55/209 (26.3%)	52/190 (27.3%)	32/126 (25.4%)
	Non-clinical	135/368 (36.7%)		103/376 (27.4%)	
Colistin (polymyxins)	Clinical	0/81 (0.0%)	4/105 (3.8%)	3/95 (3.2%)	6/63 (9.5%)
	Non-clinical	9/184 (4.9%)		17/188 (9.0%)	
Gentamicin (aminoglycosides)	Clinical	2/80 (2.5%)	4/104 (3.8%)	3/95 (3.2%)	6/63 (9.5%)
	Non-clinical	19/184 (10.3%)		12/188 (6.4%)	
Nalidixic acid (quinolones)	Clinical	40/81 (49.4%)	24/105 (22.9%)	23/95 (24.2%)	13/63 (20.6%)
	Non-clinical	60/184 (32.6%)		42/188 (22.3%)	
Tetracycline (tetracyclines)	Clinical	33/80 (41.3%)	23/104 (22.1%)	17/95 (17.9%)	19/63 (30.2%)
	Non-clinical	103/184 (56.0%)		81/188 (43.1%)	

[a] considering resistance to ciprofloxacin and nalidixic acid.

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(2017) and tetracycline (2017) in clinical isolates. In non-clinical isolates, high resistance proportions (30.0% < 50.0%) were observed in 2014 to ciprofloxacin, nalidixic acid and tetracycline. Increasing resistance was encountered to ampicillin and tetracycline in clinical isolates from 2015 to 2017. Nalidixic acid and ciprofloxacin resistance prevalence in clinical isolates decreased from 2015 to 2017. Resistance proportions lower than 13.0% in clinical and non-clinical isolates were found to colistin, cefotaxime and gentamicin.

Analyses revealed that resistance to colistin, cefotaxime and ampicillin differed significantly between clinical and non-clinical isolates. Resistance to ampicillin and colistin was less frequent in clinical isolates (ampicillin: OR and 95% CI = 0.44 (0.3–0.64), $p<0.001$; colistin: OR and 95% CI = 0.75 (0.73–0.76), $p<0.001$) while cefotaxime showed higher resistance proportions in clinical isolates with an OR>1 (OR and 95% CI = 4.58 (1.56–15.1), $p = 0.007$) (Table 5). No significant differences were encountered between clinical and non-clinical isolates for the (fluoro-)quinolones. However, differences were close to significance (OR and 95% CI = 1.30 (0.98–1.73), $p = 0.064$).

An association was found between year and resistance to colistin (OR and 95% CI = 0.94 (0.93–0.94), $p<0.001$). No significant association between year and resistance to (fluoro-)quinolones was shown. However, again the association was close to being significant (OR and 95% CI = 1.12 (0.99–1.27) $p = 0.064$). The analysis showed an association between TF of broilers with colistin and AMR (OR and 95% CI = 1.07 (1.06–1.08), $p<0.001$).

Turkeys

A total of 344 clinical isolates and 372 non-clinical isolates were collected from turkeys from 2014 to 2017. The highest resistance proportions (>50.0%) were encountered for ampicillin (2014 and 2016) and tetracycline (2014) in non-clinical isolates. In clinical isolates, highest resistance prevalence was found to ampicillin (2017). High resistance frequencies (30.0% < 50.0%) in non-clinical isolates were displayed for ciprofloxacin (2014 and 2016), nalidixic acid

Table 5. Univariate analysis results for broilers and turkeys per antimicrobial class and per (fluoro-)quinolone drug.

Antimicrobial class/ drug	Animal species	Factor	p-value	OR (CI)
Ampicillin	Broiler	AM usage	0.220	1.07 (0.96–1.2)
		Isolate type	<0.001	0.44 (0.3–0.64)
		Year	0.897	0.99 (0.84–1.16)
	Turkey	AM usage	0.792	1.01 (0.96–1.05)
		Isolate type	<0.001	0.4 (0.29–0.53)
		Year	0.857	1.01 (0.88–1.17)
Cefotaxime	Broiler	AM usage	NA	NA
		Isolate type	0.007	4.58 (1.56–15.1)
		Year	0.189	1.42 (0.85–2.47)
	Turkey	AM usage	NA	NA
		Isolate type	0.095	0.27 (0.04–1.07)
		Year	0.999	1.0 (0.54–1.84)
Ciprofloxacin	Broiler	AM usage	0.225	0.69 (0.38–1.25)
		Isolate type	0.05	1.45 (1.0–2.1)
		Year	0.028	1.2 (1.02–1.41)
	Turkey	AM usage	0.033	1.23 (1.02–1.48)
		Isolate type	0.206	0.81 (0.59–1.12)
		Year	0.028	0.84 (0.72–0.98)
Ciprofloxacin + nalidixic acid ((fluoro-)quinolones) [a]	Broiler	AM usage	0.171	0.75 (0.49–1.13)
		Isolate type	0.004	1.45 (1.12–1.88)
		Year	0.004	1.18 (1.05–1.32)
	Turkey	AM usage	<0.001	1.33 (1.16–1.52)
		Isolate type	0.527	0.93 (0.74–1.17)
		Year	<0.001	0.79 (0.7–0.88)
Colistin	Broiler	AM usage	0.023	1.82 (1.1–3.13)
		Isolate type	0.025	0.1 (0.01–0.48)
		Year	0.016	0.57 (0.35–0.88)
	Turkey	AM usage	0.034	0.82 (0.67–0.98)
		Isolate type	0.062	0.52 (0.26–1.02)
		Year	0.018	1.48 (1.08–2.06)
Gentamicin	Broiler	AM usage	0.661	1.04 (0.86–1.27)
		Isolate type	0.913	0.96 (0.45–1.93)
		Year	0.673	1.07 (0.78–1.47)
	Turkey	AM usage	0.064	4.25×10^{-6} (7.82×10^{-12}–2.05)
		Isolate type	0.035	0.5 (0.26–0.94)
		Year	0.624	0.93 (0.69–1.24)
Nalidixic acid	Broiler	AM usage	0.47	0.81 (0.45–1.45)
		Isolate type	0.041	1.46 (1.02–2.11)
		Year	0.069	1.16 (0.99–1.36)
	Turkey	AM usage	<0.001	1.47 (1.21–1.79)
		Isolate type	0.624	1.08 (0.78–1.5)
		Year	<0.001	0.72 (0.61–0.85)
Tetracycline	Broiler	AM usage	0.008	2.91 (1.32–6.46)
		Isolate type	0.007	0.55 (0.35–0.85)
		Year	0.127	0.87 (0.73–1.04)
	Turkey	AM usage	<0.001	1.68 (1.33–2.13)
		Isolate type	<0.001	0.38 (0.27–0.51)
		Year	<0.001	0.73 (0.63–0.85)

[a] considering resistance to ciprofloxacin and nalidixic acid.

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Table 6. Multivariate analysis results for broilers and turkeys per antimicrobial class and per (fluoro-)quinolone drug.

Antimicrobial class/ drug	Animal species	Factor	p-value	OR (CI)
Ciprofloxacin	Broiler	Isolate type	0.239	1.27 (0.85–1.91)
		Year	0.124	1.15 (0.96–1.37)
	Turkey	AM usage	0.997	1.0 (0.47–2.08)
		Year	0.578	0.84 (0.45–1.53)
Ciprofloxacin + nalidixic acid ((fluoro-)quinolones) [a]	Broiler	Isolate type	0.064	1.30 (0.98–1.73)
		Year	0.064	1.12 (0.99–1.27)
	Turkey	AM usage	0.920	1.03 (0.59–1.78)
		Year	0.349	0.8 (0.5–1.26)
Colistin	Broiler [b]	AM usage	<0.001	1.07 (1.06–1.08)
		Isolate type	<0.001	0.75 (0.73–0.76)
		Year	<0.001	0.94 (0.93–0.94)
	Turkey	AM usage	0.502	1.22 (0.68–2.18)
		Isolate type	0.016	0.39 (0.17–0.81)
		Year	0.129	2.31 (0.76–6.9)
Gentamicin	Turkey	AM usage	0.052	$1.2x10^{-6}$ ($9.88x10^{-13}$–1.02)
		Isolate type	0.028	0.49 (0.25–0.91)
Nalidixic acid	Broiler	Isolate type	0.150	1.34 (0.9–2.0)
		Year	0.278	1.1 (0.93–1.31)
	Turkey	AM usage	0.901	1.05 (0.44–2.4)
		Year	0.429	0.75 (0.36–1.48)
Tetracycline	Broiler	AM usage	0.144	1.97 (0.8–4.92)
		Isolate type	0.101	0.66 (0.4–1.08)
	Turkey	AM usage	0.005	97.92 (3.66–2502.81)
		Isolate type	<0.001	0.4 (0.29–0.55)
		Year	0.011	13.84 (1.76–104.98)

[a] considering resistance to ciprofloxacin and nalidixic acid.

[b] application of a different statistical method to overcome the perfect and quasi-perfect separation phenomenon in logistic regression.

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(2014) and tetracycline (2016). In clinical isolates, high resistance proportions were encountered for ampicillin (from 2014 to 2016), ciprofloxacin (2014, 2016 and 2017), nalidixic acid (2014) and tetracycline (2014 and 2017).

Resistance to ampicillin, colistin, gentamicin and tetracycline was less frequent in clinical isolates (ampicillin: OR and 95% CI = 0.4 (0.29–0.53), $p<0.001$; colistin: OR and 95% CI = 0.39 (0.17–0.81, p = 0.016; gentamicin: OR and 95% CI = 0.49 (0.25–0.91), $p = 0.028$; tetracycline: OR and 95% CI = 0.4 (0.29–0.55), $p<0.001$) (Tables 5 and 6). No significant differences were encountered between clinical and non-clinical isolates for cefotaxime. However, differences were close to significance (OR and 95% CI = 0.27 (0.04–1.07), $p = 0.095$).

Analysis showed a significant association between TF with tetracyclines and resistance to tetracycline (OR and 95% CI = 97.92 (3.66–2502.81), $p = 0.005$) and between year and resistance to tetracycline (OR and 95% CI = 13.84 (1.76–104.98), $p = 0.011$). An association between TF with aminoglycosides and resistance to gentamicin was close to being significant (OR and 95% CI = $1.2x10^{-6}$ ($9.88x10^{-13}$–1.02) $p = 0.052$).

Discussion

The main objective of this work was to compare *E. coli* AMR in clinical and non-clinical isolates from German broilers and turkeys. Furthermore, we wanted to investigate other potential

factors that may be associated with AMR in the isolates. Our hypotheses were: (1) The level of AMR is higher in clinical isolates than in non-clinical isolates and (2) there is an association between changes in AMU and changes in AMR in isolates. In order to challenge our hypotheses, we applied univariate and multivariate logistic regression analyses assessing the OR of resistance in *E. coli* from German broilers and turkeys to an antimicrobial panel (cefotaxime, ciprofloxacin, colistin, nalidixic acid, tetracycline, gentamicin and ampicillin) with the explanatory variable "isolate type"(clinical vs. non-clinical isolates). Further variables were included in the analyses: (1) year (from 2014 to 2017) and (2) TF of broilers and turkeys with antimicrobials.

The relationship between AMR and AMU has been described in livestock [25, 49, 50]. During the last years, Germany has reduced antimicrobial consumption in food-producing animals considerably [51]. In 2014 the sales figure for antimicrobials were 149.3 mg/Population Correction Unit (PCU)), while in 2017 this figure was reduced to 89 mg/PCU [4]. This reduction in the antimicrobial sales was also reflected in the TF data of broilers and turkeys [17]. Likewise, in Germany, the level of AMR in commensal *E. coli* from livestock was effectively reduced [5, 6]. Usage data collected at farm or veterinary level are required to better address the AMR assessment in livestock [51].

Therapy frequency and resistance data of isolates from broilers and turkeys in Germany were evaluated on a national level as an association of farm level was not possible with the available data. The minimum number of isolates per year and origin was defined to 25 isolates. EFSA set up a minimum of 10 isolates in their reporting system acknowledging that this number may be too low [25]. We increased the minimum number of isolates to address these concerns and ensure the reliability of the results.

To our knowledge, Germany is the only country that provided analogous public data available on national AMU per drug class and *E. coli* AMR in non-clinical isolates from both animal species (i.e. broilers and turkeys). Discussion of broilers and turkeys results is addressed below drug by drug.

Ampicillin resistance proportions from broilers remained stable in non-clinical isolates between 2014 and 2016. The percentage of ampicillin resistance in *E. coli* from broilers in Germany was similar to the EU average in non-clinical isolates in 2014 (55.7% vs. 58.6%) and in 2016 (59.3% vs. 58.0%) [5, 6, 28]. Ampicillin resistance percentage in clinical isolates was higher in 2017 than in years before. However, in the model, the year did not show a significant association with resistance to ampicillin in isolates. The model showed a higher probability of resistance in non-clinical isolates to ampicillin in broilers.

In Germany, TF of broilers with penicillins dropped sharply between 2014 and 2017. However, resistance prevalence in clinical and non-clinical *E. coli* isolates did not decrease. Similarly in France, no association between the use of penicillins and resistance to ampicillin was encountered as sales figures showed an abrupt reduction of penicillin sales from 2014 to 2017 in poultry [19], while ampicillin resistance proportions from broilers in non-clinical isolates did not change between 2014 (55.8%) and 2016 (55.9%) [5, 6]. However, data published from other European countries showed an association between the use of penicillins and resistance to ampicillin in isolates from healthy broilers [28]. In the Netherlands, resistance to ampicillin in non-clinical *E. coli* isolates from broilers decreased between 2014 (62.1%) and 2016 (47.0%) [5, 6] being in line with a reduction of penicillins use in the same time interval [52, 53]. In Denmark, ampicillin resistance in non-clinical isolates from pigs did not change from 2014 to 2017. In line with that, the use of penicillins did not change [54]. Longer periods with low TF with penicillins are likely to be required to obtain a reduction of ampicillin resistance in isolates from broilers in Germany.

Ampicillin resistance proportions in isolates from turkeys did not change significantly from 2014 to 2016. The percentage of ampicillin resistance in isolates from healthy turkeys in Germany was similar to the EU in 2014 (64.1% vs. 69.0%) and in 2016 (63.3% vs. 64.6%) [5, 6]. Similar to broilers, resistance prevalence to ampicillin from turkeys increased in 2017 in clinical isolates. However, the year as variable did not reveal any significant association with the resistance to ampicillin in isolates. The statistical analysis in turkeys provided a significantly higher probability of resistance in non-clinical isolates to ampicillin.

Therapy frequency with penicillins in turkeys decreased substantially from 2014 to 2016, but increased in 2017. Ampicillin resistance prevalence did not decrease either in clinical nor non-clinical isolates of turkeys. In France, no association was found between the reduction of sales figures in poultry from 2014 to 2017 [19] and ampicillin resistance in non-clinical isolates from turkeys in 2014 (64.3%) and 2016 (67.0%) [5, 6]. In Sweden, antimicrobials are not frequently used for bacterial disease treatments in poultry [55]. This is in line with comparatively low resistance proportions to ampicillin in 2014 (25.4%) and in 2016 (8.2%) [5, 6]. We did not find simultaneous data on the use of penicillins and on resistance in isolates to ampicillin from turkeys in other countries to help us discuss and clarify these results.

Similar to broilers, there is probably a need to keep low TF for longer periods in order to achieve a decrease of ampicillin resistance in isolates in Germany.

Resistance percentages of clinical and non-clinical isolates to ampicillin in *E. coli* tended to be higher in turkeys than in broilers. In line with that, TF of turkeys with penicillins was also higher (4 to 5 times). These differences in TF would be expected to exert significant differences in the prevalence of ampicillin resistance between broilers and turkeys. An explanation might be that a TF about six is still high enough to sustain these resistance levels.

Colistin resistance in clinical and non-clinical isolates from broilers did not change significantly over time. Higher resistance proportions in non-clinical isolates were found in Germany than in the EU average in 2014 (7.0% vs. 0.9%) and in 2016 (4.0% vs. 1.9%) [5, 6]. The model found higher resistance proportions in non-clinical isolates and associations of year and TF with resistance to colistin in isolates.

Therapy frequency of broilers with polymyxins decreased from 2014 to 2016 but increased in 2017. Colistin resistance showed a tendency to decrease in clinical (2015: 1.3%; 2016: 0.0%; 2017: 0.0%) and in non-clinical isolates (2014: 7.0%; 2016: 4.0%) although the difference was not significant. In the Netherlands, colistin resistance in isolates of *E. coli* from broilers was not observed in 2014 and 2016 (0.0%) [5, 6]. In line with that, the use of colistin was consistently very low from 2014 to 2017 [53]. In Sweden, colistin is not used in poultry and no colistin resistance was observed [5, 6]. Longer periods with low TF with polymyxins might be likely to be required to assess a major decrease of colistin resistance in non-clinical isolates.

Clinical and non-clinical isolates in turkeys showed both an increasing resistance frequency not being significant. Similar resistance proportions in non-clinical isolates were found in Germany and in the EU average in 2014 (4.9% vs. 7.4%) and in 2016 (9.0% vs. 6.1%) [5, 6]. The analysis found higher resistance odds to colistin in non-clinical isolates.

Therapy frequency with polymyxins of turkeys decreased from 2014 to 2017 while resistance to colistin in clinical and non-clinical isolates tended to increase over time. Apparently, the TF decrease with polymyxins did not reduce the prevalence of colistin resistance in isolates from turkeys. In Sweden colistin resistance was null in 2014 and 2016 [5, 6] being in line with the non-use of colistin in poultry. We did not find analogous data on the use of polymyxins and on resistance in isolates to colistin in turkeys from other countries to help us discuss and clarify these results. Similar to broilers, longer periods with low TF with polymyxins are likely required to observe a decrease of colistin resistance in isolates. Low resistance percentages might remain even after the use of colistin has ceased as it is the case with chloramphenicol

(banned in 1994 in Europe) [56]. While florfenicol, a drug from the phenicol family, may be used to treat poultry, no preparations containing the active substance are authorised for poultry in Germany [57]. Therapy frequency with polymyxins was 1.5 to 2 times higher in turkeys than in broilers. That was in line with the higher resistance proportions in isolates from turkeys.

The scientific community is concerned about colistin, an effective antimicrobial against multi-drug resistant gram-negative bacteria, because of the mobile colistin resistance (mcr) determinants discovered in isolates from humans and animals. Different *mcr*-genes have frequently been found in *E. coli* isolates from animals and food in Germany that were phenotypically resistant to colistin [58]. This together with the higher consumption of this drug in German livestock than in most other EU countries [4] may explain the tendency towards a higher prevalence of colistin resistance in isolates from poultry in Germany than in the rest of the EU. In Germany, *mcr*-1 occurs mainly in non-clinical isolates from poultry production while proportions in cattle and pig isolates are significantly lower [58]. This is not in line with reports from Asian countries where *mcr-1* is also widespread in pigs and cattle. This might reflect different AMU patterns between countries [58].

Gentamicin resistance in clinical and non-clinical isolates from broilers did not change significantly over time. Resistance proportions to gentamicin in isolates from healthy broilers tended to be lower in Germany than in the EU in 2014 (7.0% vs. 11.6%) and in 2016 (6.8% vs. 8.9%) [5, 6].

Therapy frequency with aminoglycosides of broilers decreased sharply between 2014 and 2015 but increased again from 2015 to 2017. This is not in line with gentamicin resistance percentages in clinical and non-clinical isolates across the years. However, other European figures showed opposite results [28]. In France, sales figures of aminoglycosides for poultry tended to increase from 2014 to 2017 and an increasing tendency of resistance to gentamicin in non-clinical isolates between 2014 (1.4%) and 2016 (3.2%) was observed [5, 6]. In the Netherlands, the use of aminoglycosides in broilers decreased from 2014 to 2016 [53]. In line with that, resistance to gentamicin in non-clinical isolates from broilers tended to decrease between 2014 (6.4%) and 2016 (4.3%) [5, 6]. Long periods with low TF of aminoglycosides might be likely required to cause a decrease in proportion of resistant isolates to gentamicin in Germany.

In turkeys, gentamicin resistance percentages in non-clinical isolates did not vary significantly between 2014 and 2016 and were similar to EU levels (2014: 10.3% vs. 10.0%, 2016: 6.4% vs. 6.2%) [5, 6]. The statistical analysis in turkeys provided a significantly higher probability of resistance data on non-clinical isolates to gentamicin. Resistance to gentamicin in clinical isolates did not change significantly either from 2014 to 2016, but tended to increase between 2016 (3.2%) and 2017 (9.5%).

The model found a non-significant association between small changes in TF and resistant isolate percentage. We considered this relationship close to significance an artefact.

TF with aminoglycosides of turkeys remained stable from 2014 to 2017 (1.18; 1.22; 1.22; 1.16). This was in line with gentamicin resistance proportions in clinical and non-clinical isolates across the years. In Sweden, aminoglycoside use was particularly low in livestock [4]. In line with that, no gentamicin resistance was observed for turkeys in 2014 and 2016 (0.0%) [5, 6].

Therapy frequency of broilers with aminoglycosides was 7 to 11 times higher than TF of turkeys. However, gentamicin resistance proportions of broilers and turkeys were similar in clinical and non-clinical isolates. We did not find analogous data on the use of aminoglycosides and on resistance to gentamicin in isolates from turkeys from other countries to help us discuss and clarify these results.

Gentamicin itself is not approved for use in poultry in Germany but other antimicrobials from the same family (e.g. neomycin or spectinomycin) are. Similar to gentamicin, neomycin and spectinomycin inhibit the synthesis of proteins by binding to the 30s ribosomal sub-unit causing a misreading of the DNA of *E. coli*. Dissemination of AMR genes addressing this mechanism could explain resistance proportions to gentamicin in isolates of *E. coli* from poultry [59]. Further studies are required (a) to clarify why TF differences with aminoglycosides between broilers and turkeys did not affect significantly the resistance proportions in Germany and (b) to determine whether the spread of AMR genes addressing the latter action mechanism of aminoglycosides may explain gentamicin resistance in isolates from poultry.

Third generation cephalosporins are not licensed for use in poultry in the EU and therefore differences found between cefotaxime resistance data on clinical and non-clinical isolates cannot be attributed to the use of cephalosporins. In line with not using cephalosporins in poultry, cefotaxime resistance proportions in non-clinical isolates from turkeys and broilers were very low. Resistance prevalence to cefotaxime in non-clinical isolates from broilers in Germany tended to be lower than the EU average in 2014 (1.3% vs. 5.1%) and in 2016 (1.1% vs. 4.0%), but similar for turkeys (2014: 2.2% vs. 2.3%; 2016: 2.1% vs. 2.7%) [5, 6]. Cefotaxime resistance proportions in clinical isolates from broilers remained stable from 2015 to 2017 (3.9%; 8.0%; 4.9%). Resistance to cefotaxime was more likely in clinical isolates (Table 5). Cefotaxime resistance in clinical isolates from turkeys was rare, tended to be less frequent than in non-clinical isolates (OR and 95% CI = 0.27 (0.04–1.07), $p = 0.095$) and did not change significantly from 2014 to 2017 (0.0%; 0.0%; 2.1%; 0.0%).

The ban or non-licensing of antimicrobials in food producing animals limits resistance prevalence but low resistance percentages remain. In line with that, low proportions of fluoroquinolone resistance in isolates from livestock are shown in United States [20] and in Australia [60] after the cessation of the use of fluoroquinolones.

Resistance to ciprofloxacin and nalidixic acid in non-clinical isolates from broilers was high and increased significantly between 2014 (47.8%; 44.8%) and 2016 (59.9%; 56.5%). In contrast, in the EU resistance proportions of *E. coli* isolates from broilers to ciprofloxacin and nalidixic acid did not change between 2014 (65.7%; 64.0%) and 2016 (62.6%; 59.8%) [5, 6]. In Germany, resistance proportions to these antimicrobials were lower than in the EU average in 2014. In clinical isolates from broilers, resistance percentages to ciprofloxacin and nalidixic acid decreased between 2015 (66.7%; 67.1%) and 2017 (53.7%; 46.3%) showing an opposite trend in resistance frequency to data on non-clinical isolates. A similar contrary resistance trend for fluoroquinolones as an entire family (i.e. nalidixic acid + ciprofloxacin) was found in clinical (2015: 66.9%; 2016: 60.0%; 2017: 50.0%) and non-clinical isolates (2014: 46.3%; 2016: 58.2%).

No significant differences were encountered between clinical and non-clinical isolates for (fluoro-) quinolones in general. However, differences were approaching significance (OR and 95% CI = 1.30 (0.98–1.73), $p = 0.064$).

Therapy frequency with fluoroquinolones in broilers decreased non-linearly from 2014 to 2017. Minor TF increases were encountered between 2014 and 2015 and between 2016 and 2017. This is in contrast to increasing resistance proportions to ciprofloxacin and nalidixic acid in non-clinical isolates between 2014 and 2016 but in line with decreasing resistance proportions to ciprofloxacin and nalidixic acid in clinical isolates. In France, sales figures of fluoroquinolones for poultry decreased between 2014 and 2016 [19] and resistance in non-clinical isolates to ciprofloxacin and nalidixic acid in broilers decreased accordingly between 2014 (44.2%; 42.0%) and 2016 (35.6%; 34.0%) [5, 6]. Likewise, in Netherlands, the use of fluoroquinolones in broilers decreased from 2014 to 2017 [53] and resistance to ciprofloxacin and nalidixic acid in non-clinical *E. coli* isolates from broilers tended to decrease accordingly between 2014 (47.6%; 44.6%) and 2016 (41.0%; 39.3%) [5, 6]. However, some other countries have

reported no associations between the use of fluoroquinolones and resistance to nalidixic acid in broilers [28]. Additionally, some farms without using any fluoroquinolone showed a substantial resistance prevalence to these drugs suggesting that fluoroquinolone resistance *E. coli* may be transferred onto farms via replacement [61]. Biosecurity seems to be an important influencing factor on fluoroquinolone *E. coli* resistance [61]. Longer periods with linear decreasing TF with fluoroquinolones including biosecurity level variables might be required to show a clear TF effect on resistance to ciprofloxacin and nalidixic acid in isolates in Germany. Further studies considering farm management (such as conventional vs. organic production and farms showing different biosecurity levels), molecular typing and genomic data variables are required to clarify differences in results between clinical and non-clinical isolates.

A close to significant association in broilers was found between year and resistance to (fluoro-) quinolones in isolates (OR and 95% CI = 1.12 (0.99–1.27) $p = 0.064$).

In turkeys, resistance of ciprofloxacin, nalidixic acid and the tested (fluoro-)quinolones in total tended to decrease over time. Resistance proportions to ciprofloxacin and nalidixic acid in non-clinical isolates were lower in Germany (2014: 40.8%; 32.6% and 2016: 32.4%; 22.3%) than the EU average (2014: 50.3%; 43.5% and 2016: 46.3%; 37.2%) [5, 6]. In clinical isolates, a major decrease was found to ciprofloxacin and nalidixic acid between 2014 and 2015 while levels remained stable from 2015 to 2017.

The TF with fluoroquinolones in turkeys decreased particularly from 2014 to 2016 and did not change between 2016 and 2017. This is in line with the decreasing tendency of fluoroquinolone resistance in clinical and non-clinical isolates in turkeys. Fluoroquinolones are not used in Sweden to treat poultry. In line with that, resistance proportions to ciprofloxacin and nalidixic acid were very low in 2014 (11.2%; 11.2%) and 2016 (5.7%; 6.3%) [5, 6].

Therapy frequency of turkeys with fluoroquinolones was 1.7 to 2 times higher than in broilers, while resistance in isolates tended to be higher in broilers. We did not find analogous data on the use of fluoroquinolones and resistance in isolates to gentamicin in turkeys from other countries.

The use of fluoroquinolones, highest priority critically important antimicrobials for humans, in mass medication in food producing animals is a public health concern [62]. Fluoroquinolone resistance proportions in isolates from poultry are lower in the United States, where the use of these antimicrobials is not allowed in livestock, in comparison to other large poultry producers where these drugs are approved [20].

Tetracycline resistance in non-clinical isolates from broilers tended to decrease between 2014 and 2016. Resistance proportions to tetracycline in non-clinical isolates from broilers were lower in Germany than the EU average in 2014 (33.5% vs. 50.1%) and in 2016 (27.7% vs 47.1%) [5, 6]. Resistance percentages to tetracycline in clinical isolates from broilers did not change between 2015 (17.3%) and 2016 (14.0%) but increased significantly in 2017 (31.7%).

Therapy frequency of broilers with tetracyclines decreased substantially between 2014 and 2015 and increased slightly from 2015 to 2017. This was in line with numerically decreasing resistance in non-clinical isolates [5, 6] and also with increasing resistance percentages in clinical isolates between 2016 and 2017.

In turkeys, tetracycline resistance proportions in non-clinical isolates decreased between 2014 and 2016. They were lower than in the EU in 2014 (56.0%% vs. 70.9%) and in 2016 (43.1% vs 64.8%) [5, 6]. Resistance prevalence to tetracycline in clinical isolates also decreased from 2014 (41.3%) to 2016 (17.9%), but increased again in 2017 (30.2%). The statistical analysis showed a higher probability of resistance in non-clinical isolates to tetracycline in turkeys.

Therapy frequency of turkeys with tetracyclines decreased continuously from 2014 to 2017. This is in line with the decreasing resistance in non-clinical isolates, but not with the

increasing resistance in clinical isolates between 2016 and 2017. The model identified significant associations of TF and year with resistance to tetracycline.

Therapy frequency of turkeys with tetracyclines was 5 to 10 times higher than in broilers. In line with that, resistance prevalence in turkeys was also higher, which supports the association of resistance to tetracycline in *E. coli* to use of tetracycline.

Tetracyclines are substances commonly used for the treatment of food producing animals representing around 28.0% of all sold veterinary antimicrobials in 2014 and around 26.0% in 2017 in Germany [4]. This is in line with the high resistance rates for tetracyclines that may be caused by continuous high use of the substances in the animal population [28, 32].

The differences shown between clinical and non-clinical isolates underline the necessity to have clinical and non-clinical data collection systems in place. At European level, data on non-clinical isolates are collected by the EFSA surveillance system while data on clinical isolates are not yet being collected by European institutions on a routine basis. Only the VetPath monitoring system, financed by the pharmaceutical industry, collects data on clinical isolates in livestock in Europe [11, 13, 14].

In clinical isolates, we observed an increase in resistance from 2016 to 2017 for ampicillin and tetracycline in broilers and for ciprofloxacin, nalidixic acid, ampicillin, gentamicin, tetracycline and colistin in turkeys. This might be because *E. coli* strains carrying the respective resistance genes were introduced in the animal population from other sources. In case this phenomenon in clinical isolates keeps increasing in the following years, it could diminish the differences encountered between clinical and non-clinical isolates for these substances in our study. Further explanatory variables (e.g. molecular typing or genomic data) are required to clarify this phenomenon but were not available in our study.

The sampling frames from data on clinical and non-clinical isolates differ being able to contribute to the differences encountered in this work. Data on non-clinical and clinical isolates compared in this work differed respectively in the following aspects: (a) Mandatory (non-clinical) vs. voluntary (clinical isolates) data collection basis, (b) isolate collection at the slaughterhouse vs. during the lifetime or at time of death or during post mortem, (c) isolate collection at a fixed age vs. different ages, (d) caecal samples vs. diverse sample origins and (e) data representative for the animal population in the country vs. data representative for the samples examined in the laboratories contributing to the system. The pathogenicity of the isolates tested was not determined in this study. While it can be assumed that many of the clinical isolates were avian pathogenic *E. coli* because they were isolated from diseased animals, we did not investigate these isolates beyond their phenotypic resistance to the antimicrobials. Vice versa, the commensal E. coli isolates were from healthy animals, but this obviously does not assure that they might not be pathogenic under specific circumstances. We, therefore, chose for the terminology of clinical and non-clinical isolates rather than pathogenic or non-pathogenic isolates.

There was a significant reduction in antimicrobial sales to veterinarians in Germany and likewise in TF from 2014 to 2017 in broilers and turkeys [17]. We found associations between the year (from 2014 to 2017) and resistance to colistin in broilers and to tetracycline in turkeys. However, a significant association between TF and resistance was only found for tetracycline in turkeys and for colistin in broilers (Tables 5 and 6). This suggests that other factors not considered in this study may have had a major influence on the resistance proportions. One of those might be colonization of chicks after hatching with bacteria from the hatchery environment or carry over from previous fattening flocks in the housing environment [63, 64].

We have observed partly different trends in resistance in clinical and non-clinical isolates with an identical TF. Specific *E. coli* strains could dominate in the clinical isolates due to their pathogenicity but not in the randomly selected commensals providing a plausible explanation to the differences in results reported in this work.

Further studies with longer time ranges are required (1) to clarify the differences found between clinical and non-clinical isolates and (2) to assess the long-term effects of changes in AMU and in AMR.

Conclusions

- In line with our hypothesis, resistance to cefotaxime was more frequent in clinical than in non-clinical isolates in broilers. In contrast, a higher probability of resistance in non-clinical isolates was encountered for ampicillin and colistin in broilers and for ampicillin, colistin, gentamicin, and tetracycline in turkeys. This suggests that other factors not considered in the manuscript, such as animal age at time of sample collection in clinical isolates, genetic data or sample type may have an effect on resistance prevalence.
- Due to the differences of trends and proportions shown in this study between clinical and non-clinical isolates, this work suggests that it is not enough to analyse data on either of the two to show a proper resistance proportion to a drug per an animal type within a country. Data on clinical isolates and non-clinical isolates should both be considered.
- Although the relationship between AMU and AMR is generally well documented, in our study the association of AMU of a drug class with AMR to a specific drug from this class was only significant for colistin in broilers and tetracycline in turkeys. This could suggest that is not enough to address AMR by reducing AMU indicating that as many influencing AMR factors as possible should be taken into consideration.
- Resistance rates to ampicillin and fluoroquinolones were among the highest in all populations. Resistance to tetracycline was highest in turkeys, but not in broilers in line with differences in AMU.
- The effect of the year was only found significant for resistance proportions to colistin for broilers and to tetracycline for turkeys. A decreasing association was only observed to colistin for broilers. It could suggest that longer periods with continuous low TF are required to demonstrate a resistance decrease in prevalence. However, as pointed out above, AMU reduction alone might not be enough in some cases to achieve a decrease in AMR.

Supporting information

S1 Data. Phenotypical antimicrobial resistance data of clinical and non-clinical *Escherichia coli* from broilers and turkeys in Germany between 2014 and 2017.
(XLSX)

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Chapter 5: Comparison of phenotypical antimicrobial resistance between clinical and non-clinical E. coli isolates from broilers, turkeys and calves in four European countries

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Comparison of Phenotypical Antimicrobial Resistance between Clinical and Non-Clinical *E. coli* Isolates from Broilers, Turkeys and Calves in Four European Countries

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Abstract: Livestock data on antimicrobial resistance (AMR) are commonly collected from bacterial populations of clinical and non-clinical isolates. In contrast to data on non-clinical isolates from livestock, data on clinical isolates are not harmonized in Europe. The Normalized Resistance Interpretation (NRI) method was applied to overcome the lack of harmonization of laboratory methods and interpretation rules between monitoring systems. Statistical analyses were performed to identify associations between the isolate type (clinical vs. non-clinical) and resistance to four antimicrobials (ampicillin, tetracycline, gentamicin, and nalidixic acid) per animal category in Germany and France. Additional statistical analyses comparing clinical and non-clinical isolates were performed with the available data on the same antimicrobial panel and animal categories from the UK and Norway. Higher resistance prevalence was found in clinical isolates compared to non-clinical isolates from calves to all antimicrobials included in Germany and France. It was also found for gentamicin in broilers from France. In contrast, in broilers and turkeys from Germany and France and in broilers from the UK, a higher resistance level to ampicillin and tetracycline in non-clinical isolates was encountered. This was also found in resistance to gentamicin in isolates from turkeys in Germany. Resistance differed within countries and across years, which was partially in line with differences in antimicrobial use patterns. Differences in AMR between clinical and non-clinical isolates of *Escherichia coli* are associated with animal category (broiler, calf, and turkey) and specific antimicrobials. The NRI method allowed comparing results of non-harmonized AMR systems and might be useful until international harmonization is achieved.

Keywords: AMR; clinical isolates; non-clinical isolates; broiler; turkey; calf; *E. coli*

1. Introduction

Antimicrobials are essential to maintain the human and animal health status. They allow bacterial infections, one of the most frequent disease groups in livestock, to be controlled. However, the effectiveness of antimicrobials has been reduced due to the increase in antimicrobial resistance (AMR) caused mainly by widespread antimicrobial use (AMU) in humans and animals [1].

Global, regional, and national strategies such as the Global Action Plan (GAP) on AMR of the World Health Organization (WHO) [1], the European Union (EU) One Health Action Plan against AMR [2], and national action plans (NAP) have been implemented to limit AMR development and spread. In France, the NAPs are the "plan national de réduction des risques d'antibiorésistance en médecine vétérinaire" (Écoantibio plan) in the animal sector, the "Programme national d'actions de prévention des infections associées aux soins" (PROPIAS) in the human sector, and the "plan national de santé et d'environnement" (PNSE3) in the environment sector [3]. Germany has published the "Deutsche Antibiotika-Resistenzstrategie" (DART) [4], and the UK has published the "5-year national action plan for antimicrobial resistance 2019 to 2024" [5].

Surveillance and monitoring systems on AMR and AMU in animals, a relevant pillar of NAPs, are highly important means to (a) document the situation; (b) identify trends; (c) set up the basis for risk assessment and interventions; (d) assess effects of efforts carried out; (e) associate AMU and AMR; (f) focus and target the research [6]; and (g) advise on veterinary treatments and antimicrobial stewardship [7].

Escherichia coli are Gram-negative bacteria that are commonly found as commensals in the intestinal tract of humans and animals. They are also intestinal pathogens. Resistance to antimicrobials carried by *E. coli* may be spread horizontally to other bacteria [8]. Antimicrobial resistance dynamics may be assessed by monitoring resistance in commensal *E. coli*, a widely accepted AMR indicator [9–12].

Livestock data on AMR are classically collected from (a) diseased animals (clinical data) and (b) healthy animals (non-clinical data). Epidemiological cut-off values (ECOFFs) [13] and clinical breakpoints (CBPs) [14] are used to interpret antimicrobial susceptibility testing (AST) results. Whilst ECOFFs are preferred for monitoring and surveillance objectives contrasting the wild-type and non-wild type populations, CBPs define a microorganism as susceptible, susceptible-increase exposure, or resistant depending on the probability of a therapeutic treatment succeeding [10,15].

At European level, most resistance data on non-clinical isolates from food producing animals are collected according to the Commission Implementing Decision 2013/652/EU showing, therefore, a high degree of harmonization. Some European countries also have systems collecting data from diagnostic, or clinical, submissions (e.g., France, Norway, the United Kingdom, and Germany). While the European Food Safety Authority (EFSA) collects data on non-clinical isolates on the European level, limited data on clinical isolates from diagnostic submissions from livestock are collected in Europe. Currently, only the VetPath and MycoPath initiatives funded by the pharmaceutical sector in Europe publish such data [16–18], although there is a call to launch the European Antimicrobial Resistance Surveillance network in veterinary medicine [19].

Antimicrobial resistance data on clinical and non-clinical isolates from livestock show a lack of harmonization in various aspects within and between countries in Europe. This concerns the laboratory method (e.g., disk diffusion and microdilution), the laboratory procedure, the type of data collected (quantitative vs. qualitative data), the standards used (EUCAST, CLSI, or national standards), the interpretation criteria (ECOFFs vs. CBPs), the antimicrobial panel used, and the epidemiological data on sampled animals reported [16].

Harmonized AMU data are collected as sales data in Europe from livestock as mg/population correction unit (PCU) by the European Surveillance of Veterinary Antimicrobial Consumption (ESVAC) [16]. Antimicrobial use decreased considerably in mg/PCU from 2014 to 2017 in several countries such as Germany, France [20], and the United Kingdom [21]. In Norway, antimicrobial use was extremely low [20]. Usage data on

farm level could provide a higher level of detail than sales data, addressing the association between AMU and AMR per animal and drug category more precisely [16,22,23]. Some countries have started collecting AMU at farm level [16]. However, no harmonized data collection system on AMU at farm level has been set up in Europe yet [7,16].

Studies in Estonia and Germany investigated AMR based on clinical and non-clinical isolates. The first showed on a descriptive level a higher resistance prevalence in clinical isolates in pigs and cattle [24]. However, statistical analyses carried out in the German study for broilers and turkeys showed a general pattern with lower occurrence of resistance in clinical *E. coli* isolates as compared to non-clinical [25].

To our knowledge, there is no publication statistically comparing AMR data between clinical and non-clinical isolates from national monitoring systems using different laboratory methods and procedures. Such a publication might advise political decisions to mitigate AMR in countries.

The Normalized Resistance Interpretation (NRI) method was developed to determine cut-offs statistically, using distributions of MICs or inhibition zone diameters. This procedure facilitates comparisons of AMR data from different laboratories, based on different laboratory methods and procedures [26,27].

The main objective of this work is to compare AMR data on clinical and non-clinical isolates of *E. coli* within countries in several animal categories and to describe these results across countries. The NRI method was applied to overcome the lack of harmonization in AMR regarding laboratory methods and procedures between national monitoring systems within countries.

It is plausible to expect that isolates harvested from diseased animals might carry higher levels of resistance to regular antimicrobial treatments than random isolates from healthy animals [24]. This should be studied carefully in different animal categories and countries, as it was not confirmed in the literature [25]. The hypothesis we considered in this work was that the resistance level in *E. coli* from broilers, turkeys, and calves is higher in clinical isolates than in non-clinical isolates. The counter-hypothesis is that commensals are more frequently exposed to antimicrobials, administered for other reasons, over the course of an animal's life. We assumed that the findings on broilers and turkeys in Germany are an exception [25]. In order to challenge our hypothesis, we applied univariable and multivariable logistic regression analyses comparing resistance levels in clinical and non-clinical *E. coli* isolates within countries. The year variable was also included in the analyses. Available AMU data from countries were not included in the national statistical analyses, as AMU data showed insurmountable limitations to be analytically compared to AMR. The populations reflected in the use data were not congruent with those covered by the resistance testing (e.g., AMU data from poultry vs. resistance from broilers and turkeys and resistance data from calves vs. use data in cattle in France). However, attempts to compare AMU and AMR were performed at descriptive level.

2. Materials and Methods

2.1. Data Collection and Processing

Phenotypic AMR data of *E. coli* were collected from different sources between 2014 and 2017. Caecal samples from broilers, turkeys, fattening pigs, and calves without underlying pathologies originated from the German Zoonoses-Monitoring program (ZoMo), the French antimicrobial surveillance program in healthy animals coordinated by the French Agency for Food, Environmental and Occupational Health & Safety (ANSES), the Norwegian monitoring program for antimicrobial resistance in bacteria from feed, food and animals (NORM-VET), and the UK AMR surveillance program coordinated by the Veterinary Medicines Directorate (VMD). Diagnostic submission isolates originated from different sample types from the German Resistance Monitoring of Veterinary Pathogens (GE*RM*-Vet) in the same animal categories (i.e., broilers, turkeys, fattening pigs, and calves) and from the French surveillance network for antimicrobial resistance in bacteria from diseased animals (RESAPATH) in broilers, turkeys, and calves. Additional phenotypic AMR data on clinical

E. coli isolates from different sample types originated from (a) the Norwegian monitoring program for antimicrobial resistance in bacteria from feed, food and animals (NORM-VET) for broilers, turkeys, and quails between 2015 and 2018 and (b) from the AMR scanning surveillance system in veterinary pathogens from the Animal and Plant Health Agency (APHA) supported by the VMD in the United Kingdom for broilers, turkeys, cattle, and pigs between 2014 and 2017.

Data on clinical and non-clinical isolates from Germany and Norway, and data on non-clinical isolates from France and the United Kingdom, were obtained by broth microdilution according to the ISO 20776-1 [9,21,28–31], while data on clinical isolates from France and the United Kingdom were obtained by disk diffusion, using national standards [32,33].

Regarding the latter databases, several limitations were encountered on overlaps of (a) antimicrobials, (b) animal categories and (c) time ranges across countries. As it can be observed in Figure 1, the largest coincidence of antimicrobials and animal categories between 2014 and 2017 was shown between Germany and France. For the UK the overlap with these antimicrobial/bacterial and animal population data was restricted to two antimicrobials (ampicillin and tetracycline) in broilers. In Norway, the number of clinical isolates that could be included was limited.

Combinations of isolate type (clinical vs. non-clinical), antimicrobial, animal category, country, and year (e.g., clinical resistance data for ampicillin in broilers from Germany in 2014) with fewer than 25 isolates were excluded. The study analyzed ampicillin, gentamicin, nalidixic acid, and tetracycline in broilers, turkeys, and calves. This reflects the overlap between animal categories and between the test panels in the monitoring systems on clinical and non-clinical isolates from Germany and France between 2014 and 2017. Although colistin was also tested in clinical and non-clinical isolates in Germany and France, it was not included in the analysis, as clinical resistance data from France are produced by disk diffusion, which is considered not reliable to test colistin susceptibility [34,35].

Only broiler data were considered from the UK and Norway due to the limited number of clinical isolates from the animal categories. UK data on clinical and non-clinical isolates were also statistically analyzed, but results were included separately because of the limited overlap on the antimicrobial panel (i.e., ampicillin and tetracycline) and animal categories (i.e., broiler). Resistance data on clinical isolates from Norway were limited, and therefore only univariable analyses were performed comparing data on clinical and non-clinical isolates from broilers in 2016.

For descriptive purposes, data on antimicrobial use per animal category from Germany, France, and the UK were assessed between 2014 and 2017. However, different AMU measurements are used. Moreover, the animal categories for which data are available differ between countries. In Germany, therapy frequency (TF) is used, expressing animal exposure to antimicrobials in days under treatment. In France, the Animal Level of Exposure to Antimicrobials (ALEA) is based on sales data. German and French data on use were assessed from the respective national reports [36,37]. UK data on usage of penicillin (amoxicillin and phenoxymethylpenicillin) and tetracycline antibiotic classes in the broiler sector originated from the British Poultry Council and were provided as grams of active ingredients by the VMD (data shown as kg of active ingredients in Appendix A). It was not possible to acquire AMU data by antimicrobial in all countries. Therefore, AMU data were collected at the antimicrobial class level providing uniformity. Due to the differences in the animal populations covered between AMU and AMR in France, AMU data were not included in the statistical models.

Additionally, an estimate of antimicrobial consumption from 2018 expressed as treated live weight per kg for broilers and turkeys was provided in France based on a sample of 10 volunteer producing organizations. Assuming that the differences in the use between broilers and turkeys remained stable over time, this difference was also considered in the discussion.

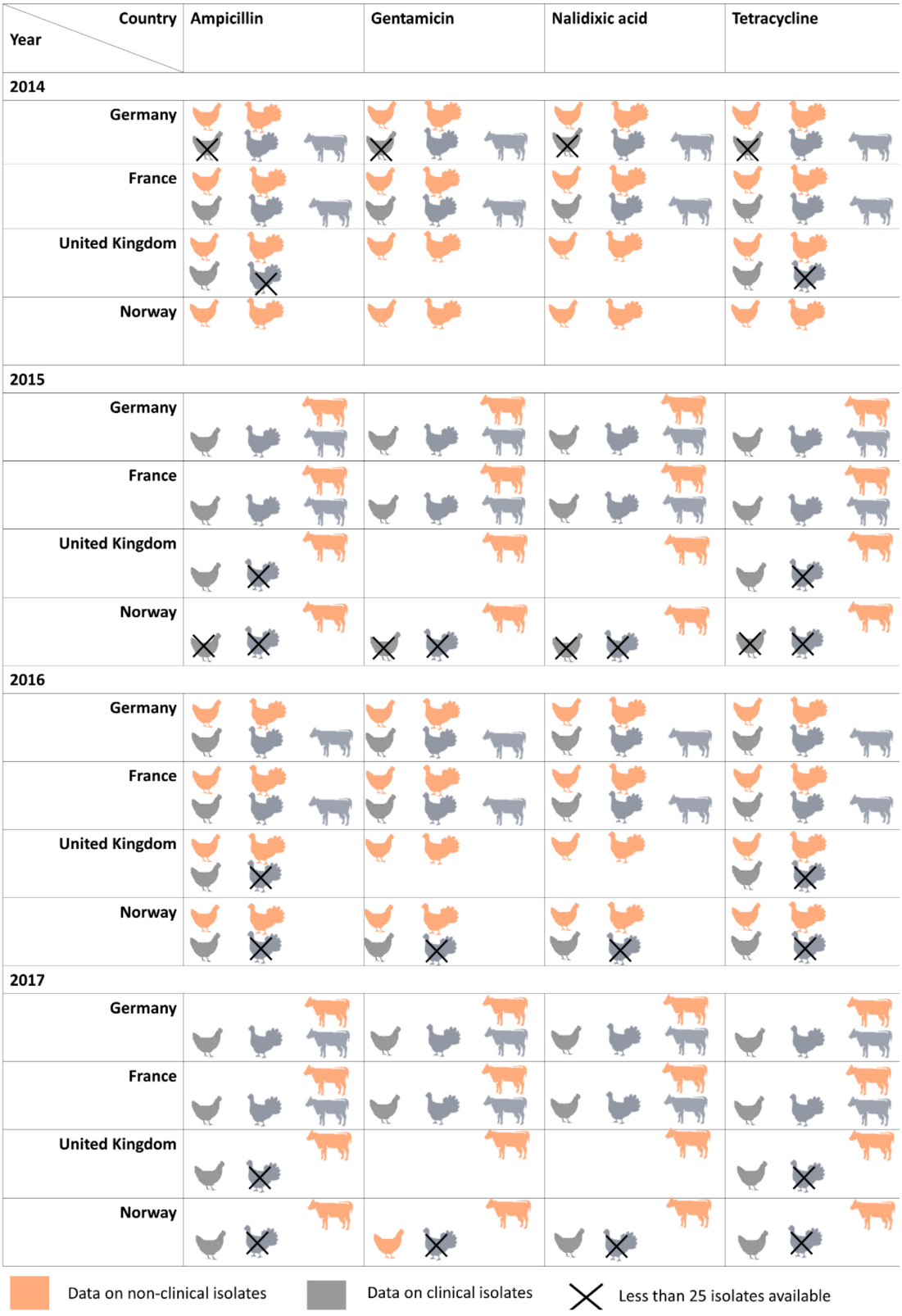

Figure 1. Data availability of broilers, turkeys, and calves in clinical and non-clinical isolates from 2014 to 2017 for ampicillin, gentamicin, nalidixic acid, and tetracycline across countries.

2.2. Overcoming the Lack of Harmonization within Countries on Antimicrobial Susceptibility Testing

Antimicrobial susceptibility tests by broth microdilution were performed according to ISO 20776-1 [9]. Data provided in this study based on broth microdilution (i.e., data on clinical and non-clinical isolates from Germany and Norway together with data on non-clinical isolates from France and the United Kingdom) were considered harmonized. Data on AST generated by disk diffusion method (i.e., data on clinical isolates from France and the United Kingdom) are based on different standards (i.e., the norm NF U 47-107 applied by the French Society of Microbiology (CASFM) and the standard of the British Society for Antimicrobial Chemotherapy (BSAC), respectively).

The NRI method assumes that data fit the normal distribution. This procedure detects the most common mode of the wild-type population (i.e., the upper part of the IZD or the lower part in a MIC distribution). Range of values covered by the normal distribution, which can be assumed to be displayed by MIC values and IZD of the wildtype isolates, are identified, calculating an objective cut-off [38]. It was applied to generate NRI cut-offs for (a) clinical isolates from Germany and Norway together with non-clinical isolates from Germany, France, the United Kingdom, and Norway; (b) clinical isolates from France; and (c) clinical isolates from the United Kingdom (Table 1). These NRI cut-offs were applied for the categorization of the AST results. NRI cut-offs were defined by the use of an Excel tool (http://www.bioscand.se/nri/ [accessed on 24 March 2021]).

Table 1. NRI cut-offs calculated and the corresponding isolates used for the determination together with broth microdilution ECOFFs from EUCAST (29 June 2020).

Antimicrobial Drug Tested	Cut-Offs (Number of Isolates Tested for the Determination)			
	Ampicillin	**Nalidixic Acid**	**Tetracycline**	**Gentamicin**
Epidemiological cut-off values (ECOFFs-EUCAST) for broth microdilution (mg/L)	>8 (73,390)	>8 (39,317)	>8 (17,276)	>2 (80,274)
French NRI cut-offs adopting all IZD data (mm)	<17 (5792)	<22 (39,317)	<20 (51,882)	<20 (55,901)
NRI cut-offs adopting all IZD data (mm) from the United Kingdom	<11 (2793)		<19 (2684)	
NRI cut-offs adopting all broth microdilution data from France, Germany, the United Kingdom and Norway (mg/L)	>16 (8381)	>8 (8379)	>4 (8373)	>2 (8372)

IZD: inhibition zone diameter; NRI: normalized resistance interpretation.

Isolates with a MIC up to the MIC NRI cut-off and with an inhibition zone diameter (IZD) above the IZD NRI cut-off (i.e., wild type isolates without acquired/mutational resistance [10]) were considered microbiologically susceptible. The other isolates were considered microbiologically resistant.

2.3. Statistical Analysis

Data were analyzed applying "the Konstanz information Miner (KNIME)" tool (Version 4.1.2) and the software "R" (Version 3.6.3) using the CRAN packages "ROCR" and "pscl". Several analyses were performed adopting different logistic regression approaches: (a) univariable and multivariable analyses for each antimicrobial (ampicillin, nalidixic acid, tetracycline, and gentamicin), each animal category (broilers, turkeys, and calves), and each country (Germany and France) were performed including the isolate type variable (clinical vs. non-clinical) and the year as independent variables to assess differences between clinical and non-clinical isolates. (b) Univariable analyses for each antimicrobial, animal category, country, and isolate type were performed including the year as an independent variable to assess resistance trends. The year in the model was analyzed as a numeric variable.

The separate analyses for the United Kingdom were restricted by the limited overlap of the antimicrobial panel (i.e., ampicillin and tetracycline; gentamicin and nalidixic acid were not covered) and animal categories (i.e., broiler) according to Figure 1. In Norway,

univariable analyses were performed per antimicrobial for broilers in 2016 including the isolate type variable as a factor in order to assess differences between clinical and non-clinical isolates. The outcome variable (i.e., susceptible (y = 0) or resistant (y = 1)) was the qualitative categorization of (a) IZD data and (b) MIC data applying the NRI method.

Univariable and multivariable models showed significant results with a *p*-value lower than 0.05. For the explanatory factor "isolate type", an odds ratio (OR) < 1 indicated a lower fraction of resistance in the clinical isolates compared to non-clinical isolates. For the explanatory factor "year", an OR < 1 indicated a decrease in resistance across the years.

3. Results

Tables 2–4 show the resistant proportions and the number of tested isolates per year and isolate type in France and Germany for broilers, calves, and turkeys and in Norway and the UK for broilers, respectively. Univariable and multivariable logistic regression results per antimicrobial and animal type within France, Germany, the United Kingdom, and Norway are shown in Tables 5 and 6, respectively. Table 7 shows univariable logistic regression analyses of the year per animal category, antimicrobial, and isolate type in France, Germany, and the United Kingdom.

Table 2. Resistant proportions applying the corresponding NRI cut-offs and numbers of clinical and non-clinical *Escherichia coli* isolates in brackets reported for broilers in Norway, the United Kingdom, France, and Germany between 2014 and 2017.

Country Drug	2014		2015	2016		2017
	Clinical	Non-Clinical [2]	Clinical	Clinical	Non-Clinical [2]	Clinical
			Norway			
AMP	na	6.3 (205)	6.3 (16) [1]	2.3 (43)	3.9 (181)	10.4 (77)
GEN	na	0.0 (205)	0.0 (16) [1]	2.3 (43)	0.6 (181)	3.9 (77)
NAL	na	3.4 (205)	0.0 (16) [1]	4.7 (43)	6.1 (181)	13.0 (77)
TET	na	1.5 (205)	6.3 (16) [1]	4.7 (43)	3.3 (181)	16.9 (77)
			UK			
AMP	52.4 (103)	73.6 (159)	56.1 (171)	34.7 (170)	79.5 (303)	26.7 (75)
TET	47.6 (103)	60.4 (159)	53.2 (171)	36.5 (170)	50.5 (303)	25.3 (75)
			France			
AMP	28.7 (411)	55.9 (227)	32.4 (519)	29.1 (515)	55.9 (188)	27.8 (421)
GEN	5.5 (1352)	1.8 (227)	6.2 (2406)	5.5 (3357)	3.2 (188)	5.1 (4156)
NAL	34.8 (881)	43.7 (227)	42.1 (1963)	47.5 (2878)	34.7 (188)	45.9 (3650)
TET	49.8 (1495)	63.4 (227)	45.8 (2638)	44.6 (3164)	62.2 (188)	45.8 (3453)
			Germany			
AMP	50.0 (18) [1]	55.2 (230)	30.3 (76)	32.0 (50)	59.3 (177)	56.1 (41)
GEN	5.5 (18) [1]	7.0 (230)	2.7 (75)	12.0 (50)	6.8 (177)	7.3 (41)
NAL	44.4 (18) [1]	44.8 (230)	67.1 (76)	58.0 (50)	56.5 (177)	46.3 (41)
TET	44.4 (18) [1]	33.5 (230)	17.3 (75)	14.0 (50)	28.8 (177)	31.7 (41)

[1] Not included in the analysis as less than 25 isolates were tested; [2] Data on non-clinical isolates are collected every two years according to the EU-legislation [9,39]; AMP: ampicillin; GEN: gentamicin; NAL: nalidixic acid; TET: tetracycline.

Table 3. Resistant proportions applying the corresponding NRI cut-offs and numbers of clinical and non-clinical *Escherichia coli* isolates reported for calves in France and Germany between 2014 and 2017.

Country Drug	2014	2015		2016	2017	
	Clinical	Clinical	Non-Clinical [1]	Clinical	Clinical	Non-Clinical [1]
			France			
AMP	79.3 (527)	84.1 (592)	53.5 (202)	83.6 (477)	83.9 (342)	45.0 (202)
GEN	23.3 (2668)	21.6 (3814)	5.9 (202)	20.9 (4543)	20.2 (4117)	4.4 (202)
NAL	47.1 (1124)	42.8 (2203)	12.4 (202)	(41.5 (2859)	35.5 (2454)	9.4 (202)
TET	79.9 (2290)	78.0 (3542)	72.8 (202)	76.2 (4323)	76.4 (3900)	65.8 (202)
			Germany			
AMP	70.9 (206)	70.5 (207)	31.8 (192)	65.3 (121)	75.0 (112)	35.5 (242)
GEN	37.9 (203)	29.9 (204)	0.5 (192)	20.7 (121)	25.9 (112)	3.3 (242)
NAL	50.5 (206)	56.6 (205)	10.4 (192)	48.8 (121)	50.9 (112)	8.7 (242)
TET	63.7 (204)	63.2 (204)	38.5 (192)	58.7 (121)	67.9 (112)	38.0 (242)

[1] Data on non-clinical isolates are collected every two years according to the EU-legislation [9,39]; AMP: ampicillin; GEN: gentamicin; NAL: nalidixic acid; TET: tetracycline.

Table 4. Resistant proportions applying the corresponding NRI cut-offs and numbers of tested clinical and non-clinical *Escherichia coli* isolates reported for turkeys in France and Germany between 2014 and 2017.

Country Drug	2014		2015	2016		2017
	Clinical	Non-Clinical [1]	Clinical	Clinical	Non-Clinical [1]	Clinical
			France			
AMP	41.6 (113)	64.4 (239)	34.8 (135)	45.9 (109)	67.0 (182)	46.2 (117)
GEN	3.8 (640)	4.2 (239)	4.3 (1188)	2.1 (1478)	1.1 (182)	1.7 (1552)
NAL	20.0 (551)	20.9 (239)	19.8 (1097)	22.2 (1426)	23.1 (182)	20.3 (1401)
TET	48.0 (783)	75.3 (239)	47.3 (1401)	41.8 (1345)	67.6 (182)	38.7 (1219)
			Germany			
AMP	37.8 (82)	64.1 (184)	36.5 (104)	37.9 (95)	63.3 (188)	57.1 (63)
GEN	2.5 (80)	10.3 (184)	3.8 (104)	3.2 (95)	6.4 (188)	9.5(63)
NAL	49.4 (81)	32.6 (184)	22.9 (105)	24.2 (95)	22.3 (188)	20.6 (63)
TET	41.3 (80)	56.0 (184)	22.1 (104)	17.9 (95)	43.6 (188)	30.2 (63)

[1] Data on non-clinical isolates are collected every two years according to the EU-legislation [9,39]; AMP: ampicillin; GEN: gentamicin; NAL: nalidixic acid; TET: tetracycline.

Table 5. Univariable logistic regression analyses per animal category and antimicrobial in France, Germany, the United Kingdom, and Norway.

Animal Category Drug	Factor	France		Germany		UK		Norway [1]	
		p-Value	OR (95% CI)	*p*-Value	OR (95% CI)	*p*-Value	OR (95% CI)	*p*-Value	OR (95% CI)
Broilers									
AMP	Isolate type	<0.001	0.34 (0.27–0.42)	<0.001	0.45 (0.31–0.65)	<0.001	0.23 (0.17–0.3)	0.628	0.59 (0.03–3.45)
	Year	<0.001	0.9 (0.83–0.97)	0.97	1.0 (0.86–1.17)	0.001	0.8 (0.7–0.92)	na	na
GEN	Isolate type	0.008	2.37 (1.33–4.77)	0.913	0.97 (0.45–1.93)	na	na	0.307	4.28 (0.16–109.86)
	Year	0.456	0.98 (0.91–1.05)	0.673	1.07 (0.78–1.47)	na	na	na	na
NAL	Isolate type	0.043	1.24 (1.01–1.51)	0.0529	1.44 (1.0–2.08)	na	na	0.72	0.75 (0.11–2.95)
	Year	<0.001	1.12 (1.08–1.17)	0.034	1.19 (1.02–1.4)	na	na	na	na
TET	Isolate type	<0.001	0.51 (0.41–0.62)	0.006	0.55 (0.35–0.83)	<0.001	0.63 (0.49–0.81)	0.673	1.42 (0.20–6.43)
	Year	0.002	0.95 (0.92–0.98)	0.172	0.89 (0.74–1.06)	<0.001	0.75 (0.65–0.85)	na	na
Calves									
AMP	Isolate type	<0.001	4.92 (3.92–6.18)	<0.001	4.66 (3.59–6.06)	na	na	na	na
	Year	0.005	0.89 (0.81–0.97)	<0.001	0.81 (0.73–0.9)	na	na	na	na
GEN	Isolate type	<0.001	4.95 (3.27–7.93)	<0.001	20.24 (10.85–43.09)	na	na	na	na
	Year	<0.001	0.94 (0.91–0.98)	<0.001	0.62 (0.53–0.72)	na	na	na	na
NAL	Isolate type	<0.001	5.65 (4.17–7.85)	<0.001	10.14 (7.18–14.67)	na	na	na	na
	Year	<0.001	0.86 (0.82–0.89)	<0.001	0.71 (0.63–0.8)	na	na	na	na
TET	Isolate type	<0.001	1.51 (1.22–1.87)	<0.001	2.79 (2.18–3.6)	na	na	na	na
	Year	<0.001	0.94 (0.9–0.97)	0.003	0.85 (0.77–0.95)	na	na	na	na
Turkeys									
AMP	Isolate type	<0.001	0.38 (0.29–0.5)	<0.001	0.4 (0.3–0.54)	na	na	na	na
	Year	0.441	0.96 (0.85–1.08)	0.857	1.02 (0.88–1.17)	na	na	na	na
GEN	Isolate type	0.892	0.96 (0.55–1.85)	0.035	0.51 (0.27–0.94)	na	na	na	na
	Year	<0.001	0.72 (0.62–0.84)	0.624	0.94 (0.7–1.25)	na	na	na	na
NAL	Isolate type	0.598	0.94 (0.74–1.2)	0.628	0.93 (0.68–1.27)	na	na	na	na
	Year	0.635	1.02 (0.96–1.09)	<0.001	0.76 (0.65–0.88)	na	na	na	na
TET	Isolate type	<0.001	0.31 (0.25–0.38)	<0.001	0.38 (0.28–0.51)	na	na	na	na
	Year	<0.001	0.83 (0.78–0.87)	<0.001	0.74 (0.64–0.86)	na	na	na	na

[1] Univariable analyses for Norway were performed per antimicrobial for broilers only in 2016 including only the isolate type variable as a factor; AMP: ampicillin; GEN: gentamicin; NAL: nalidixic acid; TET: tetracycline.

Table 6. Multivariable logistic regression analyses per animal category and antimicrobial in France, Germany, and the United Kingdom.

Animal Category Drug	Factor	France		Germany		UK	
		p-Value	OR (95% CI)	*p*-Value	OR (95% CI)	*p*-Value	OR (95% CI)
Broilers							
AMP	Isolate type	<0.001	0.34 (0.27–0.43)			<0.001	0.23 (0.17–0.3)
	Year	0.533	0.98 (0.9–1.06)			0.005	0.81 (0.7–0.94)
NAL	Isolate type	0.382	1.1 (0.9–1.35)	0.236	1.28 (0.86–1.91)	na	na
	Year	<0.001	1.12 (1.07–1.16)	0.142	1.15 (0.96–1.36)	na	na
TET	Isolate type	<0.001	0.52 (0.43–0.64)			<0.001	0.64 (0.5–0.83)
	Year	0.037	0.97 (0.93–1.0)			<0.001	0.75 (0.66–0.86)
Calves							
AMP	Isolate type	<0.001	5.03 (3.97–6.39)	<0.001	4.85 (3.66–6.47)	na	na
	Year	0.492	1.04 (0.94–1.15)	0.481	1.05 (0.93–1.19)	na	na
GEN	Isolate type	<0.001	4.86 (3.21–7.78)	<0.001	17.23 (9.11–37.02)	na	na
	Year	0.002	0.95 (0.91–0.98)	0.017	0.83 (0.71–0.97)	na	na
NAL	Isolate type	<0.001	5.49 (4.05–7.64)	<0.001	9.82 (6.82–14.45)	na	na
	Year	<0.001	0.87 (0.83–0.9)	0.584	0.97 (0.85–1.1)	na	na
TET	Isolate type	<0.001	1.48 (1.19–1.83)	<0.001	2.82 (2.15–3.71)	na	na
	Year	<0.001	0.94 (0.9–0.97)	0.877	1.01 (0.9–1.14)	na	na
Turkeys							
TET	Isolate type	<0.001	0.34 (0.27–0.42)	<0.001	0.41 (0.3–0.56)	na	na
	Year	<0.001	0.87 (0.82–0.92)	0.002	0.79 (0.68–0.92)	na	na

AMP: ampicillin; GEN: gentamicin; NAL: nalidixic acid; TET: tetracycline.

Table 7. Univariable logistic regression analyses of the year per animal category, antimicrobial, and isolates type in France, Germany, and the United Kingdom.

Animal Category Drug	Factor	France		Germany		UK	
		p-Value	OR (95% CI)	*p*-Value	OR (95% CI)	*p*-Value	OR (95% CI)
Broilers							
AMP	Clinical isolates	0.496	0.97 (0.89–1.07)	0.011	1.67 (1.13–2.49)	<0.001	0.64 (0.53–0.77)
	Non-clinical isolates	0.984	1.0 (0.83–1.22)	0.407	1.09 (0.9–1.33)	0.148	1.18 (0.94–1.48)
GEN	Clinical isolates	0.166	0.95 (0.88–1.03)	0.218	1.6 (0.76–3.46)	na	na
	Non-clinical isolates	0.351	1.36 (0.72–2.7)	0.944	0.99 (0.67–1.46)	na	na
NAL	Clinical isolates	<0.001	1.13 (1.09–1.18)	0.086	0.72 (0.49–1.05)	na	na
	Non-clinical isolates	0.061	0.83 (0.68–1.01)	0.011	1.3 (1.06–1.58)	na	na
TET	Clinical isolates	0.038	0.97 (0.93–1.0)	0.108	1.47 (0.92–2.35)	<0.001	0.7 (0.58–0.84)
	Non-clinical isolates	0.801	0.98 (0.8–1.2)	0.315	0.9 (0.73–1.11)	0.043	0.82 (0.67–0.99)

Table 7. *Cont.*

Animal Category Drug	Factor	France		Germany		UK	
		p-Value	OR (95% CI)	*p*-Value	OR (95% CI)	*p*-Value	OR (95% CI)
Calves							
AMP	Clinical isolates	0.079	1.11 (0.99–1.24)	0.804	1.03 (0.88–1.2)	na	na
	Non-clinical isolates	0.091	0.85 (0.7–1.03)	0.410	1.09 (0.9–1.34)	na	na
GEN	Clinical isolates	0.002	0.95 (0.92–0.98)	0.003	0.79 (0.67–0.93)	na	na
	Non-clinical isolates	0.503	0.86 (0.55–1.34)	0.078	2.56 (1.09–11.04)	na	na
NAL	Clinical isolates	<0.001	0.87 (0.83–0.9)	0.745	0.98 (0.85–1.13)	na	na
	Non-clinical isolates	0.339	0.86 (0.63–1.18)	0.539	0.91 (0.66–1.26)	na	na
TET	Clinical isolates	<0.001	0.94 (0.91–0.98)	0.778	1.03 (0.89–1.19)	na	na
	Non-clinical isolates	0.132	0.85 (0.69–1.05)	0.911	0.99 (0.82–1.21)	na	na
Turkeys							
AMP	Clinical isolates	0.209	1.12 (0.95–1.32)	0.036	1.26 (1.02–1.55)	na	na
	Non-clinical isolates	0.578	1.06 (0.87–1.3)	0.867	0.99 (0.8–1.22)	na	na
GEN	Clinical isolates	<0.001	0.72 (0.61–0.85)	0.089	1.57 (0.95–2.7)	na	na
	Non-clinical isolates	0.08	0.51 (0.2–1.0)	0.173	0.77 (0.53–1.12)	na	na
NAL	Clinical isolates	0.618	1.02 (0.95–1.1)	0.003	0.71 (0.57–0.89)	na	na
	Non-clinical isolates	0.596	1.07 (0.85–1.35)	0.033	0.79 (0.64–0.99)	na	na
TET	Clinical isolates	<0.001	0.87 (0.82–0.92)	0.062	0.81 (0.64–1.01)	na	na
	Non-clinical isolates	0.081	0.83 (0.67–1.03)	0.017	0.78 (0.64–0.96)	na	na

AMP: ampicillin; GEN: gentamicin; NAL: nalidixic acid; TET: tetracycline.

Note that data available for the year variable differed between data on clinical (for broilers from 2015 to 2017 and for calves and turkeys from 2014 to 2017) and non-clinical isolates (for broilers and turkeys: 2014 and 2016; and for calves: 2015 and 2017) describing trends over different time frames.

3.1. Ampicillin

Lower resistance levels were encountered in clinical isolates compared to non-clinical isolates for broilers and turkeys in France and Germany and for broilers in the UK. In contrast, higher resistance levels in clinical isolates as compared to non-clinical isolates were found for calves in France and Germany (Tables 5 and 6). In Norway, resistance levels in clinical and non-clinical isolates from broilers did not differ significantly in 2016 (Table 5).

Resistance levels increased over time in clinical isolates from broilers and turkeys in Germany but decreased in clinical isolates from broilers in the UK and in non-clinical isolates from calves in France (Table 7).

3.2. Gentamicin

Lower resistance odds in clinical isolates than in non-clinical isolates were encountered for turkeys in Germany (Table 5). Resistance levels were higher in clinical than in non-clinical isolates from broilers and calves in France and in isolates from calves in Germany (Tables 5 and 6). In Norway, resistance levels in clinical and non-clinical isolates from broilers did not differ significantly in 2016 (Table 5).

Decreasing resistance levels across the years were encountered in clinical isolates from calves and turkeys in France and from calves in Germany (Table 7).

3.3. Nalidixic Acid

Analyses revealed higher resistance levels in clinical than in non-clinical isolates from calves in France and in Germany (Table 5). However, resistance levels in clinical and non-clinical isolates from broilers were similar in 2016 in Norway (Table 5).

In broilers, the model found increasing resistance to nalidixic acid in clinical isolates in France and in non-clinical isolates in Germany over time. Decreasing resistance was detected in clinical isolates from calves in France and in clinical and non-clinical isolates from turkeys in Germany (Table 7).

3.4. Tetracycline

Lower resistance levels were encountered in clinical than in non-clinical isolates from broilers in the UK and from broilers and turkeys in France and Germany. For calves, the analysis revealed higher resistance levels in clinical isolates than in non-clinical isolates in Germany and France (Tables 5 and 6). In Norway, resistance levels in clinical and non-clinical isolates from broilers were similar in 2016 (Table 5).

Resistance levels to tetracycline decreased in clinical isolates from all animal categories in France across the years. In Germany, resistance levels decreased in non-clinical isolates for turkeys. In the UK, resistance levels decreased in clinical and non-clinical isolates from broilers (Table 7).

4. Discussion

Our hypothesis was that clinical isolates could be at higher risk of AMR than non-clinical isolates. Our study showed that this is not always the case. In Germany and France, higher resistance levels in clinical isolates were encountered to ampicillin, gentamicin, nalidixic acid, and tetracycline for calves. In broilers, this was only detected for gentamicin in France. In contrast, resistance to ampicillin and tetracycline was less likely in clinical isolates than in non-clinical isolates from broilers and turkeys in France and Germany and from broilers in the UK. This was also found for gentamicin in isolates from turkeys in Germany. Our data showed a general pattern with a higher AMR risk in clinical isolates from calves and in non-clinical isolates from broilers and turkeys, which was contrary to our hypothesis. The data analyzed had at least 41 isolates per year, isolate type, and country. The rest were discarded as they had less than 25 isolates, which is the isolates number used in a previous work as a minimum [25]. The latter threshold was based on the fact that EFSA applied a minimum number of 10 isolates to run analyses acknowledging that this number could be too low [40].

To our knowledge, this is the first study comparing resistance between clinical and non-clinical isolates of *E. coli* from different animal populations within several countries using the NRI method to harmonize results of different methods of resistance testing. A previous statistical study based on EUCAST epidemiological cut-off values with the same data on German broilers and turkeys found the same results, not confirming the hypothesis with a broader antimicrobial panel. In that previous study, additionally, lower resistance levels in clinical isolates were found to colistin for broilers and turkeys in Germany [25].

Previous studies in Estonia and Germany support our results comparing clinical and non-clinical isolates in cattle descriptively [24,41].

Resistance data from Norway did not differ significantly between clinical and non-clinical isolates to ampicillin, gentamicin, nalidixic acid, and tetracycline for broilers in 2016 (Table 2). This might be due to the limited number of clinical isolates together with the low resistance levels detected within the country.

A decreasing occurrence of resistance was found to several antimicrobials in all countries in clinical and non-clinical isolates. This indicates that effective measures are being taken in these countries to reduce resistance to antimicrobials. However, resistance increased in some animal populations to some antimicrobials.

Interestingly opposite resistance trends were observed to nalidixic acid in clinical and non-clinical isolates from broilers in countries. Resistance to nalidixic acid increased significantly in clinical isolates from broilers in France but tended to decrease in non-clinical isolates, albeit not significantly ($p = 0.061$). Conversely, in Germany, resistance to nalidixic acid decreased significantly in non-clinical isolates and tended to increase in clinical isolates from broilers in Germany, albeit not significantly ($p = 0.086$). A plausible reason for these results might be that specific strain types are associated with *E. coli* pathogens that are tested because they caused disease [42,43], while non-clinical *E. coli* isolates are randomly selected isolates, and therefore the selection basis differs [44].

Some resistance differences were found between countries. In France, resistance to tetracycline tended to be higher than in Germany in isolates from all animal categories and to ampicillin in isolates from calves. In contrast, German isolates showed higher resistance levels to nalidixic acid in isolates from all animal categories. These differences might be partially explained by differences in AMU patterns.

In France, the highest occurrence of resistance in isolates was encountered to ampicillin and tetracycline and the lowest to nalidixic acid and gentamicin for all three animal categories. This is in line with the highest level of use in poultry and cattle for penicillins and tetracyclines and the lowest for fluoroquinolones and aminoglycosides. The stratified use data for 2018 [45] indicate that the predominance of penicillins and tetracyclines was observed in both poultry species.

In Germany, differences in resistance levels were observed between the animal species. Resistance to ampicillin, tetracycline, and fluoroquinolones was high in all three animal populations. Resistance to gentamicin was only high in clinical isolates from calves. The highest occurrence of resistance in isolates from calves was found for tetracycline and ampicillin and lowest for nalidixic acid and gentamicin. This was in line with the use data. Resistance association with use was already described for broilers and turkeys [25].

In the UK, higher resistance proportions in isolates from broilers were found to ampicillin than to tetracycline, this being in line with the use data.

In Norway, the highest resistance prevalence in isolates was found for ampicillin and nalidixic acid and lower for tetracycline and gentamicin for broilers. These resistance proportions in isolates were considerably low and were in line with null or very low AMU.

In France, exposure to penicillins was higher in poultry than in cattle, while resistance was the highest in isolates from calves and the lowest in those from broilers [32]. We observed that AMU for poultry tended to decrease from 2014 to 2017 for the studied antimicrobial classes. According to the French estimate for 2018 [45], the use of penicillins was higher in turkeys than in broilers, which would be in line with the different occurrence of resistance to ampicillin. In Germany, the use of penicillins was by far the highest in turkeys. However, like in France, the highest resistance proportions to ampicillin were detected in isolates from calves, while those from broilers and turkeys were similar.

The use of penicillins in broilers in the UK decreased drastically across the years. That is in line with the decrease in resistance to ampicillin in clinical isolates from broilers. However, no decrease was observed in the non-clinical isolates.

The association between the use of penicillins and resistance to ampicillin is confirmed by data from countries with a very low total of AMU in mg/PCU from 2014 to 2017 such as Sweden or Norway that showed resistance levels low or relatively low to ampicillin (<26%)

in non-clinical isolates from broilers, turkeys, and calves [10,46–48]. This study shows that these low values are also reported for the clinical isolates from broilers in Norway.

The high resistance rates to ampicillin for calves in France and Germany may also partially be attributed to feeding waste milk from dairy cows to the calves, which has been shown to influence AMR in calves [49]. Furthermore, the type of penicillin used for treatment may be a relevant factor for the resistance development. While aminopenicillin usage has been correlated strongly with resistance to ampicillin, no association has been found between penicillin usage and ampicillin resistance in several countries [50]. Aminopenicillins affect Gram-positive and Gram-negative bacteria, promoting further resistance. Natural penicillins and related compounds [51] only work against Gram-positives and thus do not directly select for resistance in Gram-negatives.

With the exception of German broilers, the use of aminoglycosides was very low in France and Germany [32,36]. Similar low resistance prevalence was found in both countries and in Norway in non-clinical isolates for the three animal categories. Clinical isolates from calves had substantially higher resistance rates than isolates from the other categories. This higher level of resistance might not be attributed to differences in use, as this difference was small in France and in Germany. The highest use by far was observed in broilers. According to the stratified use data for 2018 [45], aminoglycoside use in broilers and turkeys was similar and very low in France. Data on AMU and AMR in France refer to different animal categories (cattle vs. calf), i.e., it is not clear, which share of the antimicrobials used in the summary category cattle are used in calves.

Resistance to gentamicin is not frequent in most animal bacteria, while resistance to streptomycin and spectinomycin is high in animal pathogens [52]. The type of aminoglycoside applied for the treatment might be a key factor in the resistance development as it has been reported for penicillins.

In Norway, the use of aminoglycosides in broilers and turkeys was negligible or non-existent. Accordingly, resistance proportions in isolates from broilers were very low in clinical and non-clinical isolates.

Fluoroquinolones are highest priority critically important antimicrobials for humans. The association between the use of fluoroquinolones and their related resistance in isolates from livestock is shown by the lower occurrence of resistance to fluoroquinolones in isolates from countries where these drugs are not licensed for use in animals or specific animal populations [40,53]. Fluoroquinolone exposure in France and Germany was higher in poultry than in cattle from France [32] and in calves from Germany [36], which is mirrored by resistance to nalidixic acid in non-clinical isolates. Higher fluoroquinolone use was observed in broilers than in turkeys in the French estimate of 2018 [45], and accordingly, resistance to nalidixic acid was higher in isolates from broilers than in those from turkeys.

In Germany, TF of calves with fluoroquinolones was the lowest. TF of broilers was 3 times higher and TF of turkeys 5 to 7 times higher. The highest resistance rates to nalidixic acid were seen in non-clinical isolates from broilers, followed by turkeys and then calves. Fluoroquinolone use for broilers and calves was in line with resistance to nalidixic acid. However, resistance to nalidixic acid in turkeys was not. The presence of quinolone resistance genes showing little or no fitness cost, such as *gyrA* [54], could make it difficult to demonstrate the relationship between the use and resistance, as non-use will not necessarily lead to a decrease in resistance.

In Norway, the use of fluoroquinolones in broilers and turkeys was null. Accordingly, resistance proportions in isolates from broilers were very low in clinical and non-clinical isolates.

Tetracyclines are frequently applied as livestock treatments representing about 28.0%, 40.5%, and 60.9% of all sold veterinary drugs in 2014 and about 26.3%, 39.3%, and 41.2% in 2017 in Germany, France, and the United Kingdom, respectively [20]. This high selective pressure for tetracycline in livestock can explain the high occurrence of resistance in clinical and non-clinical isolates of *E. coli*.

In Germany, the highest resistance to tetracycline was detected in isolates from calves and lowest from broilers, which was in line with the differences in use levels.

The use of tetracyclines in France was higher in poultry than in cattle. However, tetracycline use was higher in turkeys than in broilers according to the estimate for 2018 [45]. This agrees with resistance to tetracycline in isolates. Resistance prevalence was higher in isolates from turkeys and calves than in those from broilers.

In Norway, the use of tetracyclines in broilers and turkeys was negligible or non-existent. Accordingly, resistance proportions in isolates from broilers were very low in clinical and non-clinical isolates.

The use of tetracycline in broilers in the UK decreased drastically across the years, which is in line with decreasing resistance proportions of clinical and non-clinical isolates from broilers.

In addition to all the factors mentioned above, the presence of multi-resistant bacteria and the phenomenon of co-resistance might also explain the instances of non-agreement in trends of AMU and AMR [55,56].

Data on non-clinical isolates are routinely collected for some animal populations and food items by EFSA at the EU level, while data on clinical isolates are not commonly and routinely collected and reported to the EU-level by most EU countries. In line with previous reports [24,25], we found different occurrence of resistance in clinical and non-clinical isolates, although both are at the national population level exposed to the same level of antimicrobial use. One reasonable explanation for the differences observed between clinical and non-clinical isolates might be the different selection procedure for the strains. Specific *E. coli* strains prevail in the isolates from diagnostic submissions because of their pathogenicity [25]. In contrast, commensal *E. coli* are selected randomly from a broad range of available subtypes of *E. coli* that typically colonize the mammalian and avian gut. A further plausible reason might be that clinical samples are not randomly collected from all farms, and that farms submitting such samples may differ from other farms. However, this does not seem to have the same effect in the different animal populations studied. We found in most cases higher resistance risk in clinical isolates for calves and lower for broilers and turkeys within each country.

There are some arguments to be cautious of when interpreting differences between isolates in results. Caveats were detected comparing data on clinical and non-clinical isolates in Germany and France for broilers, turkeys, and calves: (a) type of data collection basis (i.e., mandatory; non-clinical isolates vs. voluntary; clinical isolates); (b) sample collection at the slaughterhouse, i.e., at the end of the production period vs. during the lifetime or post mortem; (c) samples from caeca (non-clinical) vs. samples from different tissues and materials (clinical); (d) data representative for the animal population in the country vs. data representative for the samples examined in the laboratories contributing to the system; (e) maximum of one sample per flock/herd and year (non-clinical) vs. possibility of more than one sample per flock/herd and year (clinical); (f) data availability in non-clinical isolates (2014 and 2016 for broilers and turkeys; 2015 and 2017 for calves) vs. in clinical isolates (from 2014 to 2017); and (g) data analyzed in one laboratory (i.e., French and UK data on non-clinical isolates together with Norwegian and German data on clinical and non-clinical isolates) vs. several laboratories (i.e., French data on clinical isolates).

Further studies with more explanatory variables such as farm management (e.g., organic vs. conventional, farm-level antimicrobial use), resistance levels of freshly hatched birds, molecular typing, genomic data, a common suitable AMU unit that represents each animal category (i.e., broilers, turkeys and calves), and longer periods are required (1) to better assess the association between AMU and AMR across and within countries and (2) to clarify the differences found between clinical and non-clinical isolates.

This study does not define the pathogenicity of the isolates. It is assumed that many of the clinical isolates are pathogenic *E. coli*. However, the resistance of these isolates to antimicrobials has only been investigated phenotypically. Similarly, *E. coli* isolates from

healthy animals are assumed to be non-pathogenic, but they might be pathogenic under specific cases.

This study underlines the need for harmonization of AMU and AMR monitoring and surveillance systems within and between countries [16] in order to continue advancing in the understanding of the AMR development and the association between AMU and AMR within and between sectors in a One Health approach. A common set of antimicrobials investigated across countries, as has been achieved for the non-clinical isolates, should also be targeted for the clinical isolates.

Associations between AMU and AMR also need to be interpreted with care. Use data are at the country level rather than regional or farm level and represent different animal categories than AMR data. Use data in France are available on cattle and poultry, while resistance data are on calves (i.e., a subgroup of cattle), broilers, and turkeys. Large differences have been identified in the use of antimicrobials between broilers and turkeys in Germany [36]. In France, separate data on AMU in broilers and turkeys were only available from an estimate in 2018 from a sample of volunteer producing organizations. They are not necessarily representative of the whole use for broiler and turkey populations in France. This suggests that AMU collected by type of production (e.g., broiler or laying hens instead of chicken), animal categories (e.g., broilers and turkeys instead of poultry), and age categories when the animal production cycle is long (e.g., calves instead of cattle) might provide a more suitable and accurate data basis to assess the association between AMU and AMR.

Direct comparisons of susceptible and resistant isolates based on different standards without using any statistical method to overcome the lack of harmonization of AMR on laboratory methodologies might be possible if the same CBPs were accidentally shown. In this study, the NRI method was applied as a straightforward approach to interpret AST results. NRI cut-offs for MIC values were calculated by using all broth microdilution data from France, Germany, the United Kingdom, and Norway for *E. coli*. Resulting NRI cut-offs and published EUCAST ECOFFs were similar. According to the results, identical cut-offs were obtained for gentamicin (2 mg/L) and nalidixic acid (8 mg/L). A difference of one dilution step in the cut-offs was estimated for ampicillin (NRI = 16 mg/L; ECOFF = 8 mg/L) and tetracycline (NRI = 4 mg/L; ECOFF = 8 mg/L). No significant differences were detected applying NRI cut-offs and ECOFFs in results (data not shown). These results together with the fact that the NRI method is an objective approach for the estimation of the wild-type populations in MIC and IZ distributions [27] encouraged us to use this method.

Our approach to calculating NRI cut-offs tried to make the best use of available data. EUCAST has a defined SOP for doing these calculations, but with the available data we could only do the calculations violating this SOP, with respect to the number of laboratories and isolates to be included [57]. Therefore, our NRI cut-offs cannot claim to be fully accurate. However, doing the calculations on different sub-sets of the data produced very similar results, which encouraged us to proceed.

Our study showed that the NRI method can be used to set a harmonized interpretative criterion in order to compare non-harmonized resistance data, provided that quantitative data across a substantial range of values are available. Therefore, the NRI method might be regularly used in veterinary medicine and in One Health studies until international harmonization of AST is achieved [26,27]. We concede that this issue might also be addressed by applying other statistical methods proposed, and further research comparing the approaches is warranted [13,58,59].

The lack of harmonization on AMU has not yet been overcome. Differences in units and in populations covered prevented the inclusion of AMU in the statistical models. Much can be gained if the requirement of Reg (EU) No. 6/2019 to collect consumption data is utilized to improve the comparability of data and populations in this area.

5. Conclusions

The Normalized Resistance Interpretation (NRI) approach, a method to identify the wild-type distribution, provides approximate epidemiological cut-offs, provided that quantitative data across a substantial range of values are available. These NRI cut-offs allow comparing quantitative results from antimicrobial resistance (AMR) systems with different levels of harmonization regarding the laboratory methods and procedures. This allowed comparing clinical and non-clinical isolates from animal categories in countries. Until AMR systems are globally harmonized, the NRI method may be considered an alternative to define interpretative values in order to compare and overcome lack of harmonization issues on AMR monitoring and surveillance systems. This method could be applied to mitigate AMR, advising political decisions.

In line with our hypothesis, a higher resistance risk was found in clinical than in non-clinical isolates from calves to all four included antimicrobials (i.e., ampicillin, gentamicin, nalidixic acid, and tetracycline) in Germany and France. In isolates from poultry, this was only found for gentamicin in broilers in France. In contrast to our hypothesis, a higher probability of resistance in non-clinical isolates was encountered for ampicillin and tetracycline for broilers in France, Germany, and the UK; for turkeys in France and Germany; and to gentamicin for turkeys in Germany. This suggests that the higher presence of resistance in one isolate type than the other (i.e., clinical or non-clinical isolates) is associated with the relationship between animal categories and the antimicrobial that might be related to how animals are treated following disease. This could also be attributable to other reasons such as co-selection or expansion of a successful clone. Resistance prevalence did not differ between clinical and non-clinical isolates from broilers in Norway in 2016. This might be due to the low number of isolates together with the low resistance prevalence in Norway for broilers.

Differences between countries were observed for specific isolate type–drug–population combinations on a descriptive level. These findings were mostly in line with differences in antimicrobial use (AMU), although comparison of these data has many caveats due to differences in sampling, reporting, in units of measurement, and granularity of available data.

Associations between AMU per antimicrobial class and AMR have been described in this work. However, data on AMU per drug for the same animal type as data on AMR may provide further information that ease identification of associations between AMU and AMR.

The analysis showed in most cases decreasing resistance trends in clinical and/or non-clinical isolates over time for the antimicrobials and animal categories studied in Germany, France, and the United Kingdom, suggesting that measures carried out against AMR including the reduction in AMU in each country have effective results.

6. Patents

The NRI method was used with permission from the patent holder, Bioscand AB, TÄBY, Sweden (European patent No 1383913, US Patent No. 7,465,559). The automatic and manual excel programs were made available through courtesy of P. Smith, W. Finnegan, and G. Kronvall.

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Data Availability Statement: The data presented in this study are openly available in the Zenodo platform (https://zenodo.org/record/4581137 [accessed on 24 March 2021]).

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Appendix A

Table A1. Penicillins and tetracyclines antibiotic class usage for broilers in the UK expressed as kg of active ingredient from 2014 to 2017.

Drug\Year	2014	2015	2016	2017
Penicillins *	16,122.4	11,209.2	8879.5	6612.2
Tetracyclines	12,446.0	6655.7	3310.4	712.0

* Penicillins = amoxicillin and phenoxymethylpenicillin.

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Chapter 6: General discussion

The aims of this thesis are to:

- Describe surveillance and monitoring systems on AMU and AMR in six European countries.
- Describe and compare resistance data of clinical and non-clinical *E. coli* isolates from livestock in countries.
- Describe the association of AMU and year with AMR.

The final goal of this work is to provide recommendations for improved “One Health” surveillance at the European level.

Description of surveillance and monitoring systems

In order to define the status on AMU and AMR in European countries, a description of monitoring and surveillance systems was performed (Mesa-Varona et al. 2020a).

AMU systems

Different data sources on AMU systems were found ranging from national sales data to prescription data. Systems reporting on prescription data adopted different units.

There is a consensus to report prescription data in an agreed unit on the medical side to the European Surveillance of Antimicrobial Consumption Network (ESAC-Net). ESAC-Net reports on Defined Daily Doses (DDD)/1000 inhabitants/ day and DDD/1000 inpatients/day in the community and hospital sectors, respectively. DDDs are obtained by dividing the weight of active ingredient prescribed in a country, based on sales, reimbursement or both data types, by the dose of a 70kg person (World Health Organization (WHO) 2021a).

It is relevant to understand the data collection of health care systems in Europe as the medical prescription could be carried out by different specialist types (general practitioner vs. specialised doctors) in sectors leading to significant dissimilarities in the data collection between countries from homologous databases. As an example, outpatient medications are commonly provided by hospitals in England, while they are usually prescribed by general practitioners at the request of secondary care doctors in Northern Ireland (Public Health Agency 2018). In such a situation the comparison on either hospital or outpatient prescription data between the two regions is very challenging. Therefore, analyses comparing AMU in humans across systems and countries should be done with care.

Reports on AMU are not always displaying the data according to the EU agreed unit. For instance, a report on AMU in primary care in Wales provided the consumption as DDD/1000 STAR-PU (STAR-PU: Specific Therapeutic Group Age-sex weightings Related Prescribing Units) instead of DDD/1000 inhabitants/day, the agreed unit (Public Health Wales 2018).
Similar to the human side, there is also consensus for livestock reporting sales data to the European Surveillance of Veterinary Antimicrobial Consumption (ESVAC) system. ESVAC reports on the antimicrobial weight per population correction unit (mg/PCU).
The agreed units (i.e. DDDs and mg/PCU) have some limitations (Mesa-Varona et al. 2020a). Sales data on the veterinary side show only a general overview of AMU and farm-level data are required for further analyses (Köper et al. 2020, Mesa-Varona et al. 2020a, Sanders et al. 2020, European Medicines Agency (EMA) 2019). Some AMUsage data have started to be submitted in 2019 to ESVAC (Mesa-Varona et al. 2020a). However, ESVAC has not reported any usage data so far. AMUsage data are accumulated at the national and regional levels showing a low level of harmonisation in Europe. These reports apply different AMU units and they do not always report on the same animal categories and antimicrobial classes.
The comparability of the AMUsage data from animals, already generated by some countries, could be improved by applying one out of the solutions proposed in the literature (Mesa-Varona et al. 2020a):

(1) Define a fully harmonised measure for AMUsage between countries and sectors in Europe.
(2) Provide freely numerator and denominator data in national reports allowing transforming one unit into another.

To this end, the following data on the veterinary side are required:

(a) The name of the active ingredient.
(b) The amount of active ingredient.
(c) The number of treated animals.
(d) The population at risk.
(e) The weight of treated animals.
(f) The time under treatment.
(g) The duration of the therapeutic effect of the active ingredient in the body.

In the literature (Sanders et al. 2020), these indicators have been also proposed to increase the harmonisation in the farm-level-AMU systems. However, even when comparable AMU data were analysed between systems within and across countries, significant limitations based on the health and husbandry conditions would arise (Sanders et al. 2020).

The implementation of the Reg (EU) No. 6/2019 on veterinary medicinal products will greatly improve the comparability of data and populations. It requires the collection of AMUsage data in a large number of animal categories at European level along with the consumption data already collected by ESVAC (article 57) (European Medicines Agency (EMA) 2020). The article 106 refers to "rules on appropriate measures to ensure the effective and safe use of veterinary medicinal products authorised and prescribed for oral administration via routes other than medicated feed, such as mixing of water for drinking with a veterinary medicinal product or as manual mixing of a veterinary medicinal product into feed and administered by the animal keeper to food-producing animals". It supports the implementation of the regulation. The Commission shall achieve a sufficiently detailed level of data on veterinary medicinal products (article 146).

However, this regulation might be improved as animal categories considered (e.g. pigs, cattle and calves) are inaccurate. Meaningful differences on AMUsage have been shown between animal subcategories not considered in the regulation (e.g. between weaning and fattening pigs or between dairy cows, beef animals and calves) (Flor et al. 2019).

On the medical side, other metrics such as days of treatment and number of prescriptions would be required to compare the AMUsage of humans and animals (Mesa-Varona et al. 2020a). This makes comparisons on AMU between countries and sectors a challenge.

AMR systems

The review of AMR systems in different sectors across countries in Europe also identified a lack of harmonisation on AMR monitoring and surveillance (Mesa-Varona et al. 2020a). Monitoring and surveillance systems on AMR collect data with a high level of variability regarding:

- The laboratory method. There are different laboratory methods to test the susceptibility of microorganisms to antimicrobials (i.e. disk diffusion, micro broth dilution and automated methods such as VITEK®).
- Laboratory methodology/procedure based on the standard selected (e.g. EUCAST and CLSI).
- The evaluation criteria (i.e. ECOFF or CBPs).
- Data reporting as quantitative (Minimum Inhibition Concentration (MIC) in mg/L / IZD in mm) or qualitative (Susceptible-Intermediate-Resistant (SIR)) data.
- Sample collection basis (voluntary, mandatory or sentinel).
- The isolate type collected (i.e. clinical vs. non-clinical).
- The sample type collected (e.g. caecal, faecal and tissue samples)

Further, collection systems on AMR often adopt specific standards and their corresponding evaluation criteria that do not cover all drug and bug combinations. Therefore, different standards and/or evaluation criteria are sometimes used for different drug and bug combinations in the same system. As an illustration, there is no CBP for tetracycline based on the EUCAST but on the CLSI standard. A work assessing the effectiveness of drug treatments that applies EUCAST CBPs could use the CLSI CBP for tetracycline to assess the effectiveness of the tetracycline treatment.

On the medical side, some data collection systems provide only qualitative data (i.e. SIR) without reporting on the standard applied as it is the case of the Second Generation Surveillance System (SGSS) that collects data from England and Northern Ireland (Mesa-Varona et al. 2020a). Further, most systems are collecting data from automated methods such as VITEK®. However, the methods applied are not reported. They show substantial differences in the results compared to the micro broth dilution method (Zhou et al. 2018). However, the VITEK 2® system, an automated method, seems to provide a relatively accurate assessment (Zhou et al. 2018, Bobenchik et al. 2015).

Data on clinical isolates from animals are not harmonised in Europe. In contrast, monitoring of AMR in non-clinical isolates from livestock and food is fully harmonised in Europe by the decision 2013/652/EU. This regulation has recently been repealed and replaced by the decision 2020/1729/EU, which applies from 1 January 2021. It allows new scientific developments to be taken into account by adapting the harmonised surveillance and reporting system for AMR in zoonotic and commensal bacteria from non-clinical isolates. This new regulation introduces the sole use of the WGS for specific types of bacteria.

There is currently no European system collecting clinical isolates of animals. However, there is a call to launch the European Antimicrobial Resistance Surveillance network in veterinary medicine (EARS-Vet) (Mader et al. 2021b). This initiative would monitor AMR in 8 animal categories and for 11 bacteria species from clinical isolates proposing three antimicrobial panels (Mader et al. 2021a). In Germany, studies were published proposing antimicrobial panels in clinical isolates of cows with mastitis, major food producing animals, cats and dogs (Werckenthin et al. 2008, Luhofer et al. 2004, Schwarz 2004). In the UK, another study proposed three antimicrobial panels to monitor AMR in 3 animal species and 8 bacteria species of animal pathogens (Teale and Borriello 2021). The choice of a harmonised panel in clinical isolates should be based on the scientific studies. This would improve the harmonisation of the antimicrobial susceptibility testing (AST) in clinical isolates.

A further step would be to improve harmonisation of AST between clinical and non-clinical isolates. Part of the data on clinical isolates could be reported by adopting the decision 2020/1729/EU. This would improve uniformity.

Due to this variability, comparing AMR in isolates is a challenge between countries and sectors from a One-Health perspective.

National and regional reports

National and regional reports are provided by most surveillance and monitoring systems. However, they are published in different languages and at different time intervals. For international comparisons, it would be preferable to have annual reports that are also provided in an agreed language.

Overlapping systems and reports in AMU and AMR

At the national and regional level, a large number of surveillance and monitoring systems on AMU and AMR in the human and livestock sectors were encountered within countries. Only a limited portion of data was reported to the European level. Some of the AMU and AMR systems collected and/or displayed the same data in different systems. As an illustration, on the medical side, in addition to the National Antibiotics Resistance Surveillance (ARS) in Germany, there is the Antibiotic Resistance Monitoring system in Lower Saxony (ARMIN) (Mesa-Varona et al. 2020a). In order to avoid overlap, it is important to plan the systems properly and to improve the use of the available resources. Reducing the number of overlapping systems should be addressed to improve the efficient use of resources. However, some overlap between systems seems to be useful for systems and data validation where this cannot be provided by ring trials. For instance, in Germany, the AMU data from the Hi-Tier system (HIT), the Quality and Safety GmbH system (QS) or the Sentinel project Veterinary Consumption of Antibiotics (VetCab) may be used to verify the results of the other systems results for some animal species (Mesa-Varona et al. 2020a).

The overlap encountered between systems is mirrored by reports providing the same data originating from different systems. Some reports are not based on a specific system (e.g. GERMAP (Paul-Ehrlich-Gesellschaft (PEG) 2015)). They report on the same data that had been reported on by other systems previously (e.g. GE*RM*-Vet). However, the level of reported information may be different. Reports that are not based on a specific system may help give an overview on AMU and AMR in the human and animal sectors in a region, as it is the case of GERMAP in Germany.

Tools to associate AMR with AMU

A general overview on the association between AMU and AMR between and within humans and animals is provided by the JIACRA reports comparing national totals at the EU level. Two tools that compare AMU and AMR in the human sector were identified in Germany and France (ARVIA and CONSORES, respectively). These initiatives, that perform these comparisons between AMU and AMR at hospital and even ward level, supplement the JIACRA reports.

Comparing AMR data on clinical and non-clinical isolates

We compared data on clinical and non-clinical *E. coli* isolates from livestock from 2014 to 2017 (Mesa-Varona et al. 2021, Mesa-Varona et al. 2020b).

Phenotypical data on clinical and non-clinical *E. coli* isolates from Germany were the only data available within the ARDIG consortium that were harmonised concerning the laboratory method and procedure. This facilitated the statistical analysis. However, some lack of harmonisation remained. The antimicrobial panel used differed between clinical and non-clinical isolate surveillance. The epidemiological data on sampled animals and their categorization to animal populations also differed. We decided to study AMR in poultry (i.e. broilers and turkeys) first since poultry and especially the broiler meat sector is increasing its relevance as a meat source in Germany and the entire world in recent decades (FAO 2019).

Phenotypic resistance data on clinical and non-clinical *E. coli* isolates of broilers, turkeys and calves were also compared in four EU countries (i.e. Germany, France, Norway and the United Kingdom). However, here a significantly higher number of limitations was encountered. These limitations, based on the difference between systems, were:

- The antimicrobial panels used
- The data available in the time range
- The animal category reported on AMU and AMR
- The AMU units and data sources (i.e. sales data or AMUsage)
- The drug categories reported
- The laboratory methods and procedures applied

To overcome these limitations, we focussed on the overlap of the antimicrobial panels, animal categories and data available between 2014 and 2017 across systems and countries. Data on a common antimicrobial panel (i.e. ampicillin, gentamicin, nalidixic acid and tetracycline) were reported for three animal categories (i.e. broilers, turkeys and calves) and for both isolate types (i.e. clinical and non-clinical) between 2014 and 2017 in Germany and France. UK data showed a limited overlap on the antimicrobial panel (i.e., ampicillin and tetracycline) and animal categories (only data on broilers available). Data from Norway were limited on the animal categories (only broilers) and the data available over time (i.e. only 2016 data available). We found no approach to overcome the lack of harmonisation on AMU across countries. Therefore, AMU was only included in the analysis in the national study in Germany (Mesa-Varona et al. 2021).

Different approaches were suggested to compare resistance data based on different laboratory methods and procedures. The easiest approach to address the lack of harmonisation in AMR regarding the laboratory methods and procedures is to compare directly susceptible and resistant isolates based on different standards provided that the same CBPs for the same laboratory method (i.e. broth microdilution or disk diffusion) were accidentally used. However, it does not seem to be a proper approach as CBPs differ in most cases and even the same CBP may express something different when derived with a different laboratory procedure.
Different statistical methods have been proposed in the literature to overcome this issue such as Bayesian models, error rate-bounded and modified error rate-bounded approaches and the Normalized Resistance Interpretation (NRI) method (Jaspers et al. 2016a, Jaspers et al. 2016b, Kahlmeter 2015, Valsesia et al. 2015, Jaspers et al. 2014, Kronvall 2010, Turnidge et al. 2006, Annis and Craig 2005, Kronvall et al. 2003, Craig 2000, Brunden et al. 1992, Metzler and DeHaan 1974). All statistical approaches show some subjectivity as a prior agreed standard deviation has to be set (Kahlmeter 2015). The NRI method has been defined as a valid approach for the estimation of the wild-type populations in MIC and IZD distributions (Contreras-Lynch et al. 2017, Callens et al. 2016, Kronvall 2010). In addition, EUCAST proposes explicitly the NRI method (Kronvall 2010, Kronvall et al. 2003) and the ECOFFINDER tool (Turnidge et al. 2006) as alternative approaches to define the MIC-based ECOFFs (Kahlmeter 2015). The ECOFFINDER tool method only allows estimating MIC-based ECOFFs while the NRI method also allows estimating IZD-based ECOFFs.
Since our dataset contained MIC and IZD data, we decided to apply the NRI approach to overcome the lack of harmonisation in AMR regarding laboratory methods and procedures between monitoring systems. The NRI method works with a normal distribution curve. It uses the low zone side slope or the upper zone size slope of the MIC or IZD distribution, respectively, to identify the wild-type distribution. This method requires a substantial range of values that includes the whole wild-type distribution.

In both studies, we compared data on AMR in clinical and non-clinical *E. coli* isolates. We expected that diseased animals might carry higher resistance levels in bacteria to regular antimicrobial treatments compared to those from healthy animals. However, this was only found in isolates of calves. In contrast, higher resistance levels were mainly found in non-clinical isolates of broilers and turkeys. The reason for this non-agreement remains unclear. However, it might be that other factors not analysed in this study such as genetic data, animal age at time of sample collection in clinical isolates (Gaire et al. 2021, Catry et al. 2003) and the sample type (Mohammed et al. 2018, Braykov et al. 2016) have a major influence on AMR.

Resistance prevalence did not differ between clinical and non-clinical isolates of broilers from Norway in 2016. This might be due to the low number of isolates together with the low resistance prevalence in Norway in isolates from broilers.

We also investigated AMR changes over time in both studies. Associations between year and AMR per animal type and antimicrobial were investigated in the study at the national level considering clinical and non-clinical isolates together. However, in the international study, these associations were analysed independently per isolate type. Due to that, results differed between studies. These associations were very limited in the national study. In the international study, the analyses showed in most cases decreasing resistance trends in clinical and/or non-clinical isolates overtime for the antimicrobials and animal categories studied in the countries suggesting that measures implemented against AMR including the reduction of AMU in the countries have effective results.

We analysed statistically the associations of changes in AMU with changes in AMR of isolates from poultry in the national study considering clinical and non-clinical isolates together. We expected to find a correlation between AMU and AMR as associations between AMU and AMR are well-documented (Ceccarelli et al. 2020, EFSA/EMA/ECDC 2017). However, this association was only partially shown. This could be partly due to having considered AMR in clinical and non-clinical isolates together as sometimes clinical and non-clinical isolates showed different or even opposite trends (Mesa-Varona et al. 2021, Mesa-Varona et al. 2020b).

In the international study, the association between AMU and AMR was only analysed descriptively across countries. Resistance levels differed between countries for specific combinations of isolate type – antimicrobial – population. These results were mostly in line with the differences in antimicrobial use between animal categories. Comparability of these data was limited, mainly because of dissimilarities in the granularity of available data, sampling, reporting and in the units of measurement.

Both studies had similar sources of bias. The sampling frames from data on clinical and non-clinical isolates differ. The samples collected for the non-clinical isolates originate from a representative sampling of random animals across the country. This mandatory sample collection was regulated by Decision 2013/652/EU. The clinical isolates by definition are never random as they can only come from diseased animals and they are only collected if the person in charge decides for collecting a sample and having it tested for AMR. Therefore, this sample collection is voluntary. That way, animals with recurrent disease or after unsuccessful treatment may be more likely to be sampled. Both factors might be associated with a higher

degree of resistance in the isolates. On the other hand, specific veterinarians may be sampling more frequently to avoid using too much or the wrong types of antimicrobials. This might be associated with a lower level of resistance in the isolates. Unfortunately, this background information is not collected and therefore it is difficult to interpret the differences. Targeted studies should try to collect this kind of information to better understand the data.

The age of the animal at sampling (Gaire et al. 2021, Catry et al. 2003) and the sample type (Mohammed et al. 2018, Braykov et al. 2016) have been shown to have an effect on AMR. A study showed that AMR genetic profiles change very fast after *E. coli* leaves the animal intestine (Barrera et al. 2019) revealing differences in these isolates even between faecal und caecal samples. Healthy animals are monitored collecting the same sample type (i.e. caecal), at a fixed age (i.e. at slaughter) in Europe. In contrast, diseased animals are monitored collecting different sample types (based on the disease), at different ages (based on the time of onset of the disease). This causes sample types and age of animals to vary addressing to a higher level of bias.

In the study across countries, the data interpretation was performed according to the generated NRI cut-offs trying to make the best use of available data. The Standard Operating Procedure (SOP) (European Committee on Antimicrobial Susceptibility Testing 2019) of EUCAST defines the procedure to calculate the cut-offs based on the broth microdilution method. To the best of our knowledge, there is no SOP based on the disk diffusion method. We tried to apply all SOP requirements to MIC and IZD data. With the available data, we could only partially follow this SOP. Two SOP requirements were violated:

(a) A minimum of five laboratories should provide data. This requirement was only met by French data on clinical isolates. The clinical and non-clinical isolates from the other countries (i.e. Norway, Germany and the UK) and non-clinical isolates from France were tested in a single laboratory.

(b) The modes of the individual wild-type laboratory distributions, that form the overall dataset, must be equal to or within one two-fold dilution step of the dataset distribution mode. Further, a minimum of 100 AST results and at least 15 Wild-Type isolates are required per laboratory distribution. As there is no SOP for IZD, we considered that laboratory distribution modes should be within 2 mm of the dataset distribution mode. Therefore, each laboratory should provide modes within 2 mm of the dataset distribution mode and at least 100 AST results with 15 Wild-Type isolates. This requirement was not met by French data on clinical isolates.

Due to these limitations, NRI cut-offs calculated cannot claim to be fully accurate. However, we were encouraged to proceed as calculations on different sub-sets of the data produced very similar results.

Main conclusions and recommendations

The final goal of this work is to provide recommendations for improved “One Health” surveillance at the European level.

On AMR

Collection systems on AMR often adopt specific standards and their corresponding evaluation criteria that do not cover all drug and bug combinations. Therefore, different standards and/or evaluation criteria are sometimes used for different drug and bug combinations in the same system. These systems show a lack of harmonisation within and between countries across the human and animal sectors.

Data collecting systems on AMR should collect data:

- applying the same standard (e.g. EUCAST or CLSI).
- using the same evaluation criteria (i.e. epidemiological cut offs or clinical breakpoints (or both in parallel)
- from both isolate types (i.e. clinical and non-clinical isolates)
- using a similar antimicrobial panel
- on isolates from the same sample type
- originating for the same animal categories

These requirements in the same isolate type should be complied in order to compare, evaluate and analyse AMR. Otherwise, data in most cases could not be directly compared.

The NRI approach is a statistical method that can be applied to overcome the lack of harmonisation on laboratory methods and methodologies. This statistical method requires quantitative data across a substantial range of values for the interpretation of data. Therefore, reports on AMR should provide quantitative values rather than data on the SIR level. Differences in results between automated AST methods and the micro broth dilution have been reported. Some automated systems have shown to be more accurate than other (e.g. VITEK 2®). Therefore, in case an automated method is used, the specific method should be reported.

Significant dissimilarities were found between data on clinical and non-clinical isolates within animal categories and countries. Higher resistance proportions were mainly encountered in non-clinical isolates of broilers and turkeys and in clinical isolates of calves. This underlines that AMR data on clinical and non-clinical isolates need to be evaluated separately and preferably both should be collected.

The lack of AMR harmonisation on further issues such as the lack of harmonisation on antimicrobial panels and animal categories remains. It is necessary to define a harmonised antimicrobial panel in clinical isolates and between clinical and non-clinical isolates.
Our analyses were applied in *E. coli* isolates of livestock between 2014 and 2017. However, this work could be extended in the future to other bacteria and for longer periods.

On AMU

On the medical side, it is significantly important to understand the data collection of the health care systems implemented in each country since the data collection procedure may vary. Therefore, data comparisons should be done with care. EU agreed units are not always provided in reports that show data from regional and national monitoring and surveillance systems on antimicrobial consumption in Europe. These units should always be applied.

ESVAC collects sales data from livestock in Europe using the agreed unit mg/PCU. However, this measurement only provides a general overview. Farm-level data are required for further analyses. However, there is no agreed unit for AMU in the animal sector. To overcome this issue reusing the already collected data, two different approaches have been suggested:

1. A harmonised unit for AMU
2. Numerator/denominator data to transform one unit into another:
    a. The name of the active ingredient
    b. The amount of active ingredient
    c. The number of treated animals
    d. The population at risk
    e. The weight of treated animals
    f. The time under treatment
    g. The duration of the therapeutic effect of the active ingredient in the body

The implementation of the Reg (EU) No. 6/2019 on veterinary medicinal products will greatly improve the comparability of AMU data in a large number of animal categories.
AMU data collection should be provided by drug, animal species (e.g. broilers and turkeys instead of poultry), type of production (e.g. broiler or laying hens instead of chicken), age categories when the animal production cycle is long (e.g. weaning or fattening pigs instead of pigs) and the mixture of age category and production (e.g. calves instead of cattle).
Reports on AMU should always report on the same drug and antimicrobial drug categories for the same animal category that should likewise be defined in a harmonised way.

On AMU and AMR

Availability of harmonised usage data for animals will allow enhancing the analyses on the association of use and resistance across countries.

In the field of AMR in non-clinical isolates, EFSA drove the harmonisation of EU data-collection. However, there is no incentive to harmonise AMU and AMR systems on clinical isolates. In the absence of EU reporting, there is no requirement to harmonise. A large number of AMU and AMR collecting systems were found per country and sector. Some overlap between monitoring and surveillance systems on AMU and AMR was encountered. This overlap may be convenient for system and data validation. However, a better use of the available sources could save resources.

Overlap between reports has been encountered as a logical consequence of the presence of overlap between systems. AMU and AMR reports are published in different languages and time ranges. They should be provided annually in one international language to ease published data access.

Antimicrobial use was identified as an influencing factor on AMR. However, AMR prevalence might not necessarily decrease to some drugs if only the reduction of AMU is addressed, due to phenomena such as co-resistance and multidrug resistance. The AMR crisis must be addressed from all available angles (e.g. hygienic measures, vaccination, support of scientific studies and reduction of AMU) and as a collaborative action between countries.

Summary

Description of surveillance and monitoring systems on antimicrobial use (AMU) and antimicrobial resistance (AMR) in European countries and comparison of antimicrobial use and resistance data on clinical and non-clinical isolates from livestock in countries

The objective of this work was to: (a) Describe surveillance and monitoring systems on AMU and AMR in European countries. (b) Describe and compare resistance data of clinical and non-clinical *E. coli* isolates from livestock in countries. (c) Describe the association of AMU and year with AMR. (d) Provide recommendations for improved “One Health” surveillance at the European level.

A literature search was conducted in 2018 to identify relevant peer-reviewed articles and reports on AMU and AMR. It was used for identifying and assessing monitoring and surveillance systems on AMU and AMR in the human and animal sectors and foodborne AMR in six European countries (Germany, France, Spain, The Netherlands, The United Kingdom and Norway). Logistic regression analyses were performed comparing phenotypical data on clinical and non-clinical *E. coli* isolates of several animal categories from 2014 to 2017 at national and international level. AMU was only included in the national study as we found no approach to overcome the lack of harmonisation on AMU across countries. The Normalized Resistance Interpretation (NRI) method, a statistical approach, was applied to overcome the lack of harmonization on the laboratory methods and procedures in the international study.

This work identified overlaps on monitoring and surveillance systems on AMU and AMR in the human and animal sector. A lack of harmonisation was encountered on: (a) Antimicrobial usage for livestock and, therefore, between humans and livestock and (b) AMR in clinical isolates of livestock and, therefore, between clinical and non-clinical isolates within livestock and between isolates of humans and livestock.

In both statistical studies, we expected that diseased animals might carry higher resistance levels in bacteria to regular antimicrobial treatments compared to those from healthy animals. However, this was only found in isolates of calves. In contrast, higher resistance levels were mainly found in non-clinical isolates of broilers and turkeys. This phenomenon remains unclear. However, it might be that other factors not analysed in this study such as genetic data, animal age at time of sample collection in clinical isolates and the sample type have a major influence on AMR.

Resistance prevalence did not differ between clinical and non-clinical isolates of broilers from Norway in 2016. This might be due to the low number of isolates together with the low resistance prevalence in Norway in isolates from broilers.

We also investigated AMR changes over time in both studies. Associations between year and AMR per animal type and antimicrobial were investigated in the study at the national level considering clinical and non-clinical isolates together. However, in the international study, these associations were analysed independently per isolate type. Due to that, results differed between studies. These associations were very limited in the national study. In the international study, the analyses showed in most cases decreasing resistance trends in clinical and/or non-clinical isolates over time for the antimicrobials and animal categories studied in the countries suggesting that measures carried out against AMR including the reduction of AMU in the countries have effective results.

We analysed statistically the associations of changes in AMU with changes in AMR of isolates from poultry in the national study considering clinical and non-clinical isolates together. We expected to find a correlation between AMU and AMR. However, this association was only partially shown. This could be partly due to having considered AMR in clinical and non-clinical isolates together as sometimes clinical and non-clinical isolates show different or even opposite trends.

In the international study, the association between AMU and AMR was only analysed descriptively across countries. Resistance levels differed between countries for combinations of isolate type – antimicrobial – population. These results were mostly in line with the differences in antimicrobial use between animal categories. Comparability of these data was limited, mainly because of dissimilarities in the granularity of available data, sampling, reporting and in the units of measurement.

This work proposes a list of recommendations for improved “One Health” surveillance. A One Health regarding the surveillance and monitoring of AMR and AMU is currently not straightforwardly achievable because of the lack of harmonisation of AMU and AMR surveillance within the livestock sector and also between the human and livestock sectors.

Zusammenfassung

Beschreibung der Überwachungs- und Monitoringsysteme zum Antibiotikaeinsatz (AMU) und im antimikrobiellen Resistenz (AMR) in europäischen Ländern und Vergleich der Daten zum Einsatz von antimikrobiellen Mitteln und zur Resistenz bei klinischen und nicht-klinischen Isolaten von Tieren in den Ländern

Das Ziel dieser Arbeit war es: (a) Überwachungs- und Monitoringsystemen zu AMU und AMR in europäischen Ländern zu beschreiben, (b) Resistenzdaten von klinischen und nicht-klinischen *E. coli*-Isolaten aus Nutztieren in verschiedenen Ländern zu beschreiben und zu vergleichen und dabei andere verfügbare Faktoren (d. h. das Jahr und den Antibiotikaeinsatz) einbeziehen. (c) den Zusammenhang von AMU- und dem Jahr mit AMR zu untersuchen, (d) Empfehlungen für eine verbesserte "One Health"-Überwachung auf europäischer Ebene abzugeben.

Im Jahr 2018 wurde eine Literaturrecherche durchgeführt, um relevante peer review Artikel und Berichte zu AMU und AMR zu identifizieren. Sie diente der Identifizierung und Bewertung von Monitoring- und Überwachungssystemen zu AMU und AMR im Humanbereich und in der Tierhaltung sowie zu AMR in Isolaten aus Lebensmitteln in sechs europäischen Ländern (Deutschland, Frankreich, Spanien, Niederlande, Vereinigtes Königreich und Norwegen).

Es wurden logistische Regressionsanalysen durchgeführt, um phänotypische Daten zu klinischen und nicht-klinischen *E. coli*-Isolaten verschiedener Tierkategorien von 2014 bis 2017 auf nationaler und internationaler Ebene zu vergleichen. Daten zum Antibiotikaeinsatz wurden nur in der nationalen Studie berücksichtigt, da wir keinen Ansatz gefunden haben, um die fehlende Harmonisierung der AMU-Daten zwischen den Ländern zu überwinden. Die Methode der Normalisierten Resistenz-Interpretation (NRI), ein statistischer Ansatz, wurde angewendet, um die fehlende Harmonisierung der Labormethoden und -verfahren bei der Resistenztestung in der internationalen Studie zu überwinden.

Diese Arbeit identifiziert Überschneidungen bei Monitoring- und Überwachungssystemen zu AMU und AMR im Human- und Tierbereich. Ein Mangel an Harmonisierung wurde festgestellt in Bezug auf: (a) den Einsatz von antimikrobiellen Mitteln bei Nutztieren und daher zwischen Mensch und Nutztier und (b) AMR in klinischen Isolaten von Nutztieren und daher zwischen klinischen und nicht-klinischen Isolaten innerhalb von Nutztieren und zwischen Isolaten von Mensch und Nutztier.

In beiden statistischen Studien erwarteten wir, dass Isolate von kranken Tieren höhere Resistenzwerte gegenüber eingesetzten antimikrobiellen Substanzen aufweisen könnten als

solche von gesunden Tieren. Dies wurde jedoch nur bei Isolaten von Kälbern gefunden. Im Gegensatz dazu wurden höhere Resistenzniveaus hauptsächlich in nicht-klinischen Isolaten von Masthähnchen und Puten gefunden. Die Ursache dieses Phänomens bleibt unklar. Es könnte jedoch sein, dass andere Faktoren, die in dieser Studie nicht analysiert wurden, wie z. B. genetische Daten, das Alter der Tiere zum Zeitpunkt der Probenentnahme bei klinischen Isolaten und der Probentyp einen großen Einfluss auf die AMR haben.

Die Resistenzprävalenz unterschied sich nicht zwischen klinischen und nicht-klinischen Isolaten von Broilern aus Norwegen im Jahr 2016. Dies könnte auf die geringe Anzahl von Isolaten zusammen mit der niedrigen Resistenzprävalenz in Norwegen bei Isolaten von Masthähnchen zurückzuführen sein.

Wir untersuchten auch Veränderungen der Antibiotikaresistenz über die Zeit in beiden Studien. Assoziationen zwischen Jahr und AMR pro Tierart und antimikrobiellem Mittel wurden in der Studie auf nationaler Ebene unter gleichzeitiger Berücksichtigung klinischer und nicht-klinischer Isolate durchgeführt. In der internationalen Studie wurden diese Assoziationen getrennt pro Isolattyp untersucht. Aus diesem Grund unterschieden sich die Ergebnisse zwischen den Studien. Die Assoziationen waren in der nationalen Studie sehr begrenzt. In der internationalen Studie zeigten die Analysen in den meisten Fällen abnehmende Resistenztendenzen bei klinischen und/oder nicht-klinischen Isolaten im Laufe der Zeit für die untersuchten antimikrobiellen Substanzen und Tierkategorien in den Ländern. Dies deutet darauf hin, dass die in den Ländern durchgeführten Maßnahmen gegen AMR, einschließlich der Reduzierung des Antibiotikaeinsatzes, wirksame Ergebnisse haben.

Wir analysierten statistisch die Assoziationen von Änderungen der AMU mit Änderungen der AMR von Isolaten aus Geflügel in der nationalen Studie, wobei klinische und nicht-klinische Isolate zusammen betrachtet wurden. Wir erwarteten, eine Korrelation zwischen AMU und AMR zu finden. Dieser Zusammenhang wurde jedoch nur teilweise gezeigt. Dies könnte teilweise darauf zurückzuführen sein, dass wir AMR in klinischen und nicht-klinischen Isolaten zusammen betrachtet haben, da klinische und nicht-klinische Isolate manchmal unterschiedliche oder sogar entgegengesetzte Trends zeigen.

In der internationalen Studie wurde der Zusammenhang zwischen AMU und AMR länderübergreifend nur deskriptiv analysiert. Die Resistenzniveaus unterschieden sich zwischen den Ländern für Kombinationen aus Isolattyp - Antibiotika - Population. Diese Ergebnisse standen größtenteils im Einklang mit den Unterschieden in der Verwendung von antimikrobiellen Mitteln zwischen den Tierkategorien. Die Vergleichbarkeit dieser Daten war eingeschränkt, vor allem aufgrund von Unterschieden in der Granularität der verfügbaren Daten, der Probenahme, der Berichterstattung und in den Maßeinheiten.

In dieser Arbeit wird eine Liste von Empfehlungen für eine verbesserte "One Health"-Überwachung vorgeschlagen. Eine "One Health" in Bezug auf die Überwachung und das Monitoring von AMR und AMU ist derzeit nicht ohne Weiteres erreichbar, da die AMU- und AMR-Überwachung innerhalb des Tierhaltungssektors und auch zwischen dem Human- und dem Tierhaltungssektor nicht harmonisiert ist.

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O. Mesa-Varona, B-A. Tenhagen. (2019): Surveillance and monitoring systems for antimicrobial usage in livestock animals in six European countries. In: Quantification, Benchmarking and Stewardship of Veterinary Antimicrobial Usage (AACTING) 2nd International Conference, July 2nd-3rd 2019, Bern, Switzerland. Poster.

O. Mesa-Varona, B-A. Tenhagen. (2019): Evidences of overlapping between antimicrobial resistance and drug usage surveillance and monitoring systems in the Human, Animal and Food Sectors in European countries. In: Zoonosen symposium, October 16th-18th 2019, Berlin, Germany. Poster.

O. Mesa-Varona, B-A. Tenhagen. (2019): Reviewing antimicrobial resistance and drug usage surveillance and monitoring systems in the Human, Animal and Food Sector in European countries. In: The Predoc symposium, November 11th 2019, Berlin, Germany. Poster.

O. Mesa-Varona, B-A. Tenhagen. (2021). Comparison of antimicrobial use and resistance data on clinical and non-clinical isolates from livestock in four countries. In: Junior Scientist Zoonoses Meeting. June 3rd-4th 2021. Online. Poster.

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O. Mesa-Varona, B-A. Tenhagen. (2019). Reviewing antimicrobial resistance and drug usage surveillance and monitoring systems in the Human, Animal and Food Sector in European countries. In: The 1st One Health EJP Annual Scientific Meeting, May 22nd-24th 2019, Dublin, Ireland Republic. Oral communication.

O. Mesa-Varona, R. Mader, N. Jarrige, S-A. Granier, A. Perrin, E. Jouy, C. Chauvin, H. Kaspar, M. Anjum, M. Grobbel, M. Velasova, B-A. Tenhagen. (2020): Comparison of antibiotic resistance in *Escherichia coli* from clinical diagnostic submissions and isolates of healthy

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M. Velasova, R. Smith, K. Chaintarli, **O. Mesa-Varona**, B-A. Tenhagen, H. Kaspar, R. Mader, J-P. Amat, J-Y. Madec, M-A. Anjum. (2020): Antimicrobial resistance of *Escherichia coli* isolates originating from diagnostic submissions from veterinary scanning surveillance in UK, Germany and France from 2014 to 2017. In: The 2nd One Health EJP Annual Scientific Meeting, May 27th-29th 2020, Prague, Czech Republic. Online. Oral communication.

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O. Mesa-Varona, B-A. Tenhagen. (2020): Main outputs of the work package 1 from ARDIG (Antibiotic Resistance Dynamics: the influence of geographic origin and management systems on resistance gene flows within humans, animals and the environment) project. In: The workshop on surveillance/monitoring of AMR and AMU in animals and humans, March 1st-2nd 2021, Berlin. Online. Oral communication.

Journal articles

O. Mesa Varona, K. Chaintarli, B. Muller-Pebody, M-F. Anjum, T. Eckmanns, M. Norström, I. Boone, B-A. Tenhagen. Monitoring Antimicrobial Resistance and Drug Usage in the Human and Livestock Sector and Foodborne Antimicrobial Resistance in Six European Countries. Infect Drug Resist. 2020 Apr 3;13:957-993. DOI: 10.2147/IDR.S237038.

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The authors have declared that no competing interests exist.

Declaration of independence

I hereby confirm that I have written this thesis independently. I certify that I have used only the sources and aids indicated.

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(Octavio Mesa-Varona)