THESIS INTRODUCTION

“The Knowledge Era”

Lisbon, capital of Portugal, will be in the XXI Century History, naming an important Treaty and an ambitious Agenda anchored on Knowledge, and predicting a brand new Strategy for Europe.

The “Lisbon Agenda” (2000), was initially a ten-year “development strategic plan” to convert the EU’s economy into the “most competitive and dynamic knowledge based economy in the world”. It was intended to streamline information, research and development, as an incentive for urged structural reforms, in order to reinforce competitiveness and innovation and to support eventual adjustments of the European policies to the actual society needs. However the necessary mechanisms for its implementation have failed and the “Lisbon Agenda” was replaced by the “Strategy 2020”. In the meantime, the “Lisbon Treaty” (2009) provided the European Union (EU) the tools to meet the European citizen’s highest expectations in future, setting a special emphasis on the well-being concept, prioritizing research for development, and building a European Area with free circulation of scientific knowledge and technologies.

The European Veterinary Pharmaceutical Industry (IFAH-Europe) has promptly supported the “Lisbon Agenda”, recognizing the urgent need of an updated legal frame for the veterinary medicines’ sector that could be itself more predictable, stable, harmonized, and shaped to strengthen competition and innovation, as main pillars of the veterinary medicines development, and market sustainability. Animals have several important roles in society and animal health is essential for a healthy public environment. Food producing animals are additionally part of the Gross Domestic Product (GDP), and therefore factually significant for the countries’ economies.
In line with the Lisbon’s Agenda roadmap, the «European Technology Platform for Global Animal Health» was developed in 2004, to identify and enhance the most effective tools against animal diseases of major importance in the world, referenced as such by national “mirror groups”, created for an overall benefit of the human and animal health, animal welfare, food quality, and access to the markets.

The new legislative proposal from the European Commission (EC) towards a “Better Regulation” for the veterinary medicines, is supposed to tempt more resources on research and stimulate interfaces between key related areas like food quality, animal diseases, surveillance systems etc.

The European Parliament (EP) also issued an opinion on the strategies for the European Union regarding competition, and on the relevance of scientific concepts to support such challenges, referring animal and human health, animal well-fare and human well-being as main subjects to be specifically addressed.

The EU policies on the harmonization of the Europeans rights and duties, and on the land and monetary barriers, have contributed to approximate the Member States and have surely be the baseline to a considerably long period of political stability in the region, awarding the Peace Nobel Prize, in October 2012.

Over the past five decades, the world assisted to the hastiest and most extraordinary globalization phenomenon ever, facing continuous challenges, urging innovation and prompting development, almost permanently and in a constant need of adaptation to the “modern times”. Economy’s expansion, advanced technology, demographic evolution and climatic changes are some of the world’s major occurrences that have exposed humans, animals and crops to inevitable higher risks, and have brought new defies for the human kind, consequently. One of these threats is the emergence of resistant bacteria, its
fast dissemination, and the increasing inefficiency of the existing antimicrobial medicines to treat and save, people and animals.

To all those that have lived and contributed to this remarkable technological era, with constant new medical advances, it is incredibly ironic that human beings, animals and crop’s vulnerability to microorganisms has recently re-emerged all over the world, with extreme severity. Infections are getting progressively harder to control, paradoxically due to the non-access to antimicrobial agents at all, or due to its exaggerated access and use, favouring the antimicrobial resistance phenomenon. Both scenarios exist in different countries, our days.

The responsible use of antimicrobial agents, wherever these substances are used, may be the only way out to preserve their efficacy and overthrow some serious infectious diseases that threat men and animals. Thus, it is crucial to indorse effective actions for containment of the antimicrobial resistance emergence, development and spread, supported by an adequate regulatory management, using integrated surveillance systems for both resistant bacteria and for antimicrobial agents’ consumption, in both human and animal sectors, to explore the relevant epidemiological links in between.

Human beings are to microbes, like “Goliath to David”. “Knowledge” is the best weapon men can use to keep the balance between the macro and micro world on Hearth.

For a healthy future, antimicrobials have to be preserved as humanity patrimony, for men and animals.
INTRODUCTION

Antimicrobial agents are one of most important medical advances ever [1], contributing to a significant decrease in morbidity and mortality rates, due to bacterial infections in humans and animals. Unfortunately, antimicrobial’s efficiency was always shadowed by the emergence of resistant bacteria, which can potentially affect everyone in the world, independently of being used in human or in veterinary medicine, and for that reason, antimicrobial medicines are considered societal medicines [2].

Bacteria are on Heath for more than 3.5 billions of years and less than 1% are mainly symbiotic and hosted in animals and men. The antimicrobial resistance (AMR) is also an ancient naturally phenomenon, and a bacterial survival mechanism to resist any antimicrobial agent, including those produced by other bacteria in the environment [2,3]. Antimicrobial resistance genes even predate the use of the antimicrobial medicines. Bacteria without inherent resistance to an antimicrobial may become resistant by mutation or acquiring resistance genes from other bacteria, and the resistance problem can therefore grow exponentially. Under ideal conditions, and within less of 10 hours, one only bacterium may produce an inoculum with one billion of bacteria, and considering that one spontaneous mutation occurs in about one hundred million divisions, it is extraordinary the mutation potential of a bacteria population [4,5]. Transfer of AMR genes among bacteria occurs under different mechanisms and one of the main complex consequences is the cross resistance fitness.

The more antimicrobial agents are used, the more frequently resistant bacteria are eliminated to soil, air and water, increasing the exposure of plants, people and animals, by destroying susceptible bacteria and exerting a selective pressure favouring an excessive growth of bacteria encoding resistance genes [6,7,8]. Consequently, the extensive use of antimicrobials will ultimately promote increased dissemination of
resistant bacteria strains, causing a serious hazard, which is potentially amplified by the considerable lack of new antimicrobial agents, particularly for veterinary use.

The decline in research and development of new medicines in the European Union, is mostly a market issue [9], but is also related with the pre and post marketing authorisations’ regulatory burdens for the pharmaceutical industry, which has merged into a real problem of veterinary medicine’s availability, in particular for minor species and/or minor uses (MUMS). Moreover, with the increasing emergence of the AMR, the antimicrobial medicines’ life-cycles are progressively getting shorter and insufficiently profitable to invest on other new antimicrobials, deepening the gap between infection and infection control, over the time.

After the seventy’s, out of a dark period of wars, remarkable political changes have improved people’s lifestyle and nutritional behavior in many countries, remodeling the animal production sector, in order to respond to an increased demand of animal protein. In industrial plants built nearer the rural communities and within more confined spaces, more animals were bred faster, consuming higher amounts of antimicrobials, either to prevent and treat animal diseases or to accelerate their growth [10]. Under intensive production, the grouping of animals from several origins (from out and in), without specific biosecurity and animal welfare measures, predispose to higher risks of microbial contamination and infections that need to be efficiently prevented, controlled and treated with antimicrobial veterinary medicines [11,12]. On the other hand, the transhumances of some animal species and the animal extensive production have the onus of spreading in the environment and over reasonable extensive areas, commensal, zoonotic and pathogenic microorganisms. In any case, diseased food producing animals have to be treated in time in order to limit the spread of infection and minimize the impact of productive losses, at the minimum cost possible. The clinical decisions have to be taken
promptly, based on the clinical signs, on the historic microbiota of the farm and/or of the region where the disease occurs, and also on the success rates obtained under previous similar conditions. Occasionally, a herd or a flock has even to be sacrificed to control infection.

Presently, the world’s population is increasing exponentially every day, and many climacteric, political, cultural, geophysics, and gastronomic changes are succeeding and guiding the food supply chains towards quality and safety from «stable to table». In developed countries, the quantity of food’s offer is not being regarded an issue for now, despite some embryonic concerns about it in a near future, at a global scale [13,14].

Recently, the AMR threat emerged with multi-resistant bacteria, limiting the efficiency of several antimicrobial medicines, compromising the available therapeutic options, prolonging recovery or failing to treat. According to the European Parliament, the WHO (World Health Organization) and the ECDC (European Centre for Disease Prevention and Control), the European health systems are actually facing increased expenses with resistant infections, reporting an augment of human illness and death occurrence [9], often associated with Gram-negative resistant bacteria.

There are strong evidence that modern medical, human and veterinary, practices are accelerating the emergence and spread of AMR, due to improper and exaggerated use of antimicrobial agents. Epidemiological studies have shown a consistent and statistically relevant association between consumption of certain classes of antimicrobials and AMR to those classes, urging therefore an overall reduction of antimicrobial consumption [15] considering that resistant bacteria often reveal inferiority when competing with normal bacteria in free antimicrobial environments. To reduce the actual consumption of antimicrobials both in humans and animals, those amounts must be known and closely
monitored to evaluate thereafter the impact of the management actions being taken against AMR.

The antimicrobial consumption reduction target is met when the most indispensable and responsible use is fully ensured. Any random targets or blind prohibitions, particularly on the use of veterinary antimicrobial medicines may encourage illegal uses, potentiating the toxicological risks of residues in food of animal origin and favouring AMR also.

Currently, there are only uncertain perceptions or gross estimates about veterinary antimicrobial consumption in the world [16]. In Portugal, like in many other European Union member States (EU-MS), data on sales of veterinary antimicrobials started to be collected under the ESVAC (European Surveillance of Veterinary Antimicrobial Consumption) project, envisaging data collection on antimicrobials consumption by animal species, measured and reported with harmonized technical units, comparable with the established human Defined Daily Doses (DDD). To test this part of the project, an ESVAC pilot phase on antimicrobials data consumption collection has run in pigs, within a few EU-MS. Portugal did not join the pilot phase but the same protocol was used for the present thesis, in a cross-sectional study, within five large grouped farms, located mostly in the Lisbon and Tagus Valle Region (LVTR), with approximately 1/3 of the national pig population, and with antimicrobials’ sales data reporting retrospectively to the year 2013, yielded by an exclusive supplier. The total amounts of the administered veterinary antimicrobial active substances were calculated by life cycles, according with the recommended dosage regimens, in the respective SPCs (Summary of the Product Characteristics) [17], after extrapolation to the national pig population.

Some hurdles were detected along the procedure, in line with the more recent assumption that the still ongoing ESVAC project to calculate data consumption by animal species still needs further refinement [18] to be validated. In general, this pilot phase revealed a
weak adhesion of EU-MS, and has been temporarily suspended due to regulatory, technical and funding constraints that will delay its implementation. Furthermore, the report of veterinary antimicrobials consumption in individual countries is a sensitive political issue, having as much impact in the public health as in the nation’s economy, [19] affecting trade and tourism in particular. Data accuracy is therefore a key factor in monitoring, surveillance and reporting processes. Additionally, particular attention should be given to the medicated feed which is routinely used in farms for mass medication [20], and though is still inconsistently covered by the ESVAC project, for being governed by a distinct Community legal act [21,22,23] and out of the European Medicines Agency’s jurisdiction. Furthermore, the quality controls of the authorised veterinary antimicrobial medicines and of the antimicrobial medicated feed should also be strengthened as part of an integrated post marketing surveillance system, and giving also support to the overall strategy against AMR [24].

In summary, for the benefit of human and animal health, to raise greater confidence in the European regulatory systems, ensuring transparency and exchange of accurate information that may assist science development and risk analysis on AMR, the existing and the ongoing veterinary surveillance systems on antimicrobial consumption and on resistant bacteria of animal origin, have to integrate the most possible accurate data and meet the highest precision on its outcomes.

The present thesis intends to support this whole objective, describing the regulatory aspects of the veterinary medicines which reflects the specificity of the veterinary sector, and expects to contribute for the improvement of the Community projects on integrated veterinary surveillance, particularly regarding the antimicrobial consumption, and to reinforce the one health perspective and the efficiency of the coordinated actions against AMR, detailing the veterinary traits of the antimicrobial’s usage.
PART I – REGULATORY SCOPE OF THE VETERINARY MEDICINES

1. HISTORICAL SYNOPSIS OF THE VETERINARY MEDICINES IN PORTUGAL

In the annals of the veterinary medicines in Portugal [25], «The Treasure of the Farmers», is an ancient manuscript (1762) about animal treatments by non-veterinary professionals, commonly named “alveitares”. The first volume highlights the antiquity and nobleness of agriculture and reports a range of different anatomic features in animals; the second one describes the forty seven diseases identified by a famous farmer at the time, while the third was just an update with forty eight chapters of new diseases; the fourth and last volume included several “interesting answered questions”, about the virtue and quality of the medicines´ active substances, known as “simplices”.

In the same year, the first Veterinary School in the world was created in Lyon, by initiative of a lawyer, who joined the most competent doctors and surgeons that were known at the time, to abandon the ancestral empiricism on veterinary education and start a new era for the veterinary medicine [25].

Just some decades before, major discoveries related with vaccines and antibiotics had radically changed the medical science. Pasteur and his veterinarian disciples noted that a culture of avian cholera, left abandoned near a laboratory oven, had lost its virulence and infective ability, getting immunity fitness [25]. Sometime later, the Penicillin activity was found also from a casual laboratory incident, when an antimicrobial action was observed on mold that had contaminated dirty petri dishes. Hundreds of years before Alexander Fleming, also in the cold north region of Portugal, the “Transmontan” people had already discovered the magical power of the “penícilios” to fight infections in the excoriations of hunting dogs paws, after walking and running in the asperities of the soils, through the
rough alleys of the mountains, and that were treated with the mold of the corn bread leftovers, forgotten next to the fireplace at home [25].

In Portugal, the veterinary medicine’s education would be decreed in 1855 only, in a very close model to the actual one, where pharmacology, pharmacy and veterinary law, were already part of the academic curriculum [25].

1.1 From History to the Regulatory Framework

From a common root, human and veterinary medical sciences evolved separately driven by the distinct social roles that men and animals have on Hearth. The same dichotomy separated the regulatory path of the medicinal products for human or for veterinary use.

In a recent past, after the Portugal adhesion to the EEC (European Economic Community) in 1986, veterinary medicines were governed separately as “pharmacological veterinary medicines”, and “veterinary immunological medicines”. Previous designations, such as “Veterinary medicines”, “medicines to animals”, “specialized medicines”, “products to be used in veterinary medicine”, “serums, vaccines and products to be used in veterinary therapy” all referred once, in ancient national legal acts, to the current definition of veterinary medicinal products.

The oldest known legislation on “medicines to animals” regarding those “of immunological nature”, was published in 1913, and created the “Livestock Health Services” to promote and control the use of vaccines, serums and similar products in animals, and the “Laboratory of Veterinary Pathology and Bacteriology” to manufacture those products and develop diagnostic agents [26,27]. The special importance of vaccines in the prevention of severe epizooties in food producing animals is hence well reflected in the national legislation since ever.
In 1922, the “veterinary pharmacological medicines” [28], were given specific guidance for packaging and labeling, to include already the commercial name, the indications, the manufacture name, the shelf-life, and the sentence «For Veterinary Use», (kept until our days) and was later ruled by the pharmaceutical legal act, between 1929 and 1968 [29,30]. It was only in 1931, when inspections on trade of the “specialized medicines” were reinforced [31,28], that a marketing procedure for products to be used in veterinary medicine was determined, complementing the existing legal provisions for vaccines, serums and complement products, used in veterinary therapy.

Soon after, considering the need to supply livestock with safe biological products, with high degree of purity and therapeutic value against the existing zoonosis, with proved efficacy and without any prices aggravation, new specific provisions regarding manufacture, sales, inspections and imports for veterinary immunological medicines were also published [32].

Between the thirty´s and the eighty´s, a lack of new legal provisions might be related to the political regimen in Portugal and to the slight development of veterinary medicines also, but thereafter, the awareness of the concept of residues as chemical hazards, among other foodstuffs polluting forms increased and the “use of chemical substances, drugs or medicines leaving residues in animals” was regulated for the first time [33]. All those substances, would be regulated after 1987 as veterinary medicines [34,35], with the exception of the veterinary pesticides and disinfectants that were covered by specific legislation [36]. The animal health and the public health concerns were expanded to protect the consumer´s health, by guarantying safety food of animal origin, in a new legal frame related to the use of chemical substances, drugs and medicines with prophylactic, curative or other ends that could leave residues in the animals’ organs and tissues for human consumption. It was the beginning of a new era for the veterinary medicines,
assuming the likelihood of toxicological risks from residues in food of animal origin with potential impact in human health, making the marketing authorisations applications more complex for the pharmaceutical industry and implying long-lasting and expensive studies in food producing animals. It marked also the beginning of the veterinary medicines availability crisis in the EU.

The Residues surveillance Programmes on meat, milk, eggs and honey were created under specific regulation for approval and marketing authorisation of medicinal products for veterinary use, defining withdrawal periods to be observed, attending to the specificities of pesticide substances [33] that could also be carried by animal feed. The “Nacional Plan for Residues Detection” (actual “National Plan of Residues Control”), was the first surveillance system to analyse veterinary medicine´s residues in food producing animals and in foodstuff of animal origin and therefore considered an important “food security milestone”. Food safety incompliances may be judged as public crime in Portugal.

Maximum Residues Limits (MRLs) were fixed by individual countries or adopted from those fixed by the Codex Alimentarius, which in practice was an obstacle to trade and for controls, not ensuring consumer´s health protection. In the meantime, the use of innumerous substances, drugs or medicines in animals got so much importance that a Community regulation [37,38], was published with a harmonized procedure for the establishment of MRL of veterinary medicines in foodstuff of animal origin.

Regarding the use of substances or products with hormonal effects the insufficiency of the available legal mechanisms to discipline that use, urged Community provisions [34, 39,40] to ban the use of some hormones, thireosthatics and beta-agonist substances in food producing animals, authorising though “specific use conditions” of hormonal substances (with gestagenic, estrogenic and androgenic effects), antimicrobials,
chimioterapics, antiparasitics, tranquilizers and veterinary pesticides, enforcing the veterinary prescription as well.

Over that period, there were growing concerns on how to distinguish residues of natural origin, (particularly hormones), from those arising in animals after administration of veterinary medicines. European legal acts on these matters (Hormones and MRLs) were often subject to international negotiations in order to respect the World Trade Organization (WTO) Agreement on the Application of Sanitary and Phytosanitary Measures (SPS Agreement).

Meanwhile, at national level the so called “Products for Veterinary Use” (PUV) [35] that excluded any veterinary medicine, covering many borderline products without legal scope, started to claim several indications, and more stringent requirements were published, regarding the legal provisions for its manufacture, marketing authorisation, storage, transport, market and use [41]. Shortly after, disinfectants started to be evaluated as biocides [42,43], and the veterinary pesticides, (except those not to be used directly in animals), were submitted to a reclassification procedure [44] and redefined as veterinary medicines [45,46,47,48,49]. The PUV to be administered to companion minor species (aquarium fish, ornamental and cage birds, pigeons, mail-pigeons, terrarium animals, small roedents, ferrets and companion rabbits) were also classified as veterinary medicines, subject thereafter to a simplified marketing authorisation procedure, with specific regulation on advertisement, sales and use [50]. The PUV’s regulation [51] still covers all the non-medicinal products that may add a complement value to some veterinary medicines, regulators of the animal’s physiology, and animal’s environment, cosmetics, hygiene products and in vitro diagnostic kits.
1.2 The Veterinary Medicines

The first national legal act for the veterinary medicines were published in 1987 [35,52] in accordance with the Community Directive that established Community rules for the pharmacological veterinary medicines in the EU.

In Portugal the marketing authorisation applications were “addressed” to the Health National Competent Authority (NCA) to be “submitted” to the Agriculture NCA, describing the results of the analytical, pharmacological, toxicological and clinical assays, for evaluation of the veterinary medicine’s quality, safety and efficacy files by the “Technical Commission for the Evaluation of Medicines for Veterinary Use”, assessing the influence of the veterinary medicine in human health, taking into account the proposed withdrawal periods, the therapeutic importance, the products safety, and the marketing advantages.

Until 2007, only the pharmacies, (with pharmacist property exclusivity until 2005) were authorised to the veterinary medicines retail, in accordance with the “Pharmaceutical Activity” [30,53]. “Direct acquisition” by veterinarians and agro-firms, from laboratories, importers and wholesalers, was already allowed providing that acquired medicines were to be administered to their patients or to their own animals, respectively, and always under a veterinarian’s supervision. Farm cooperatives [54] and “animal health protection groups” [55] were also qualified for direct acquisition. The classification of the veterinary medicines to be sold under prescription only (POM) started to be printed on the package labels and the advertisement rules were also regulated [30,56,57].

Later on, the administrative procedures between Health and Agriculture NCAs, became too lengthy and these were asked [58,59] to reconsider both interventions in all matters related with the evaluation and controls of veterinary medicines, though no solutions were met at that point in time.
Meanwhile, the legislation governing the veterinary immunological medicines was obsolete and required to urgently update the most important activities to be taken in prophylaxis, treatment and diagnose of the animals’ infectious diseases” [28,32,60]. The marketing authorisation procedure was revised, the manufacture and other related activities were reinforced, the legal competence of the Reference Laboratory (LNIV) maintained to authorise the introduction of any veterinary microbial strain, in national territory, and a veterinary prescription or a veterinary requisition became mandatory to sell also veterinary immunological medicines. It was also established [61,62], special requirements for assays with these medicines [63], initiating “a new era in the legal framework of the veterinary immunological medicines with quality, efficacy and safety, requiring new rules for manufacture, marketing and use [64]. Veterinary immunological medicines became part of the National Pharmacovigilance System that had just been settled to the pharmacological veterinary medicine and PUV [41]. Particular attention was given to potential interferences of immunological responses of the treated animals with the national or Community programmes for diagnose, eradication and control of animal diseases [65], strengthening the NCA, the National Reference Laboratory and the Regional Services cooperation [52,61,66,67].

The communication from NCAs to the European Medicines Agency (EMA) and to the other EU-MS, of any related national decisions, that could cause any impact on public health or animal health in third countries, was extended to the Health Word Organization and the International organization on Epizooties (OIE), which is actually the World Organization for Animal Health. The centralized procedure for marketing authorisation [68], the production and use of auto and herd vaccines were however excluded from its scope and referrals started to be
recurrent regarding the decisions to be adopted by the MS competent authorities, based on human, animal or environmental health and protection grounds”.

In the meantime, new Community legislation was published [47] and merged into only one legal code [45] the pharmacological and immunological veterinary medicines regulatory provisions that were divided since 1931.

“Pharmaceutical and immunological” veterinary medicines were finally merged into a unique national legal code [45] which determined the role of the veterinary medicines as crucial tools to promote animal health and animal welfare, and to protect public health, with considerable impact in agriculture and economy, with an “overall public benefit”[47,48].

Improving the existing marketing authorisation procedures (national, centralized, and mutual recognition), it introduced a decentralized procedure to facilitate the availability of veterinary medicines in the EU and a simplified register procedure was also created to authorise certain type of veterinary medicines, particularly those to be used in companion minor species, or on an infrequent basis. All granted marketing authorisation, formerly subject to quinquennial renewals, started to be re-evaluated only once and for an unlimited period of time, unless any pharmacovigilance issue may advice differently any time. Giving the enormous lack of availability of veterinary medicines, with potential serious consequences to animal and human health, the marketing authorisation procedure for generic veterinary medicines was also adapted, establishing data protection periods to safeguard and encourage the pharmaceutical industry to invest on investigation and development of new veterinary medicines. Labels and leaflets were also improved and a normalised veterinary prescription was legally adopted taking into account the exemption criteria for the veterinary prescription in food producing animals. Controls were reinforced by a National Plan for Control of the Use of medicines in farms, based on a
documental traceability system and data crosschecking with other official controls, particularly the residues and the animal feed control plans. Legal guidance was provided for the production and use of auto vaccines and of herd vaccines, as important tools for prevention and treatment of certain animal diseases without authorised alternatives, but only at national level.

The Actual marketing systems for the veterinary medicines are however still very different in the different EU-MS and not covered by the harmonized EU legislation. In Portugal, it was considered that the restrictive retail system in place, was an increasing factor for both veterinary medicines and food prices, potentially promoting veterinary medicine’s illegal markets. As a consequence, the legal frame of the veterinary medicine’s supply chain was altered, and the “pharmaceutical act” as well [67], withdrawing from the pharmacist sphere, the exclusivity of the veterinary medicines sales. The decision was taken on the current “qualified person” rules [46,47,70], which highlight the competition importance among liberal professions, in the EU economic reform. Thus, in the actual veterinary medicine’s national legal code [45,46,48,71], Wholesalers and retailers of veterinary medicines may have as a qualified person with technical responsibility, either a pharmacist or a veterinarian.

The revision of the Community legislation on veterinary medicines is actually ongoing upon an EC Regulation proposal, forward in September 2014. The emergence of antimicrobial resistance, the one health perspective and the veterinary medicine’s availability and access, are major concerns for the animal sector which will certainly be reflected on the forthcoming Community Regulation for veterinary medicinal products. Greater level of harmonization will be ensured by that new legal act that will substitute the current Directive, differently transposed across the EU-MS.
2. THE VETERINARY MEDICINES NATIONAL AND COMMUNITY REGULATION

The veterinary medicines are subject to a marketing authorisation procedure, at national or Community level by centralized, decentralized or mutual recognition procedures [45,46,47,52,68]. The evaluation procedure depends on the applicant’s option, except when the veterinary medicine to be authorised is already marketed in any EU-MS, which may in those cases be the reference MS for the mutual recognition or decentralised procedure, involving the MSs, where that veterinary medicine is to be marketed. The difference between mutual recognition and decentralised procedure refers mainly to the number of countries involved in the process. Norway, Liechtenstein and Island, as EFTA (European Free Trade Association) countries, are non EU-MS that voluntarily comply with the EU legislation on veterinary medicines.

Within the different EU-MS, the NCAs are distinctly organized, functioning mostly as common agencies for both human and veterinary medicines, but not in Portugal (Fig.1).

Figure 1 - Organics of the Portuguese national competent authority for the veterinary medicines (DGAV)

The European Medicines Agency (EMA) and the Food and Drug Administration (FDA), are the EU and the United States of America Medicines Agencies, respectively.
The NCAs are the regulatory entities responsible for the human and veterinary medicines in the European Economic Area countries, represented at the EMA’s Management Board and at the informal Group of the “Heads of Medicines Agencies” (HMA), whose mission is to protect and promote public health in Europe, fostering an effective and efficient European medicines regulatory system. Within a unique model for cooperation and work-sharing, HMA liaises with the EMA and with the EC in the operation of the European medicines regulatory network, addressing key strategic issues, focusing on the development, co-ordination and consistency of the European medicines regulatory system, ensuring the most effective and efficient use of available resources, providing a high level of performance to stakeholders, being trustworthy and receptive to new knowledge (http://www.hma.eu/).

The European Medicines Agency’s Committee for Veterinary Medicinal Products (CVMP) (http://www.ema.europa.eu/) is the European Scientific Committee which is responsible for preparing opinions on marketing authorisations for veterinary medicinal products, and for preparing scientific guidelines that reflect a harmonised approach of the EU Member States and the Agency on how to interpret and apply the Community Directive requirements [47,48] for the demonstration of quality, safety, efficacy and environmental impact. It helps the applicants or marketing authorisation holders to prepare their marketing authorisation applications or variations.

The EU harmonization process is already substantial in the veterinary medicines sector but still not enough to decline additional guidance from the existing Guidelines and from Notice to Applicants [72]. The actual legislative act governing the veterinary medicines in Europe is a Directive that just sets out the goals that all EU-MS must achieve, leaving up to the individual countries to devise their own laws on how to reach those goals, which cause some obstacles to the alignment with some European projects like the ESVAC.
Full harmonization on veterinary medicines is therefore envisaged on the ongoing revision procedure at the Council, by republishing that Directive as a Regulation, which is already a binding legislative act that must be applied in its entirety, across the EU.

2.1 Veterinary Medicines Market and Use – National Regulation

Circulation and trade of animals and of foodstuff of animal origin, are regulated in the EU, including inspections and residues monitoring [39,40] to reduce commercial barriers, protect public health and defend consumer’s safety.

As mentioned by Briyne. N (2014), there is no single market for veterinary medicines in the EU and consequently, there are different or even insufficient authorised products in the different EU-MS [73], depending on their markets, which strengthened the need of a European Medicines Agency, to grant Community marketing authorisations [74].

In contrast with the veterinary medicines, medicated feed may circulate between MS in the EEA, whenever it contains a medicated premix with quantitative and qualitative composition similar to any authorised medicated premix in the recipient country. Trade rules for medicated feed are therefore different [21,22], despite being just a “new formulation” of the medicated premixes, which are veterinary medicines [75] subject to a veterinary prescription as well.

The veterinary medicine´s retail is still out of the scope of the EC legislative proposal for revision, which means that the EU-MS will continue to regulate it differently at national level, although in authorised channels only, that may include veterinarians, wholesalers, pharmacies and other authorised establishments, depending on the EU-MS. In Portugal, [46] with the exemption of the pharmacies, all the formerly authorised establishments had a pharmacist and a veterinarian as qualified persons, but nowadays, for economic reasons, the position is taken by one of those professionals only. The “veterinary pharmacies” have
been created as well. In any case the submission of a veterinary prescription has been always mandatory.

Until very recently, all authorised veterinary medicines were prescription only medicines (POM) in Portugal, but currently the Community prescription criteria to classify the veterinary medicines applies during the marketing authorisation procedure [47] and there are already few non POM veterinary medicines. Antimicrobials are in any case POM only. The enforcement of a veterinary normalised prescription (Fig. 2) and of a veterinary requisition, both requiring veterinarian validation (Fig. 4), and the records on veterinary medicines’ administrations in farms are actually the basis of the control plan to monitor the use of medicines in food producing animals, as a national project.

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**Figure 2** – Normalised veterinary prescription national model
Whereas in many third countries, there are only simple recommendations for the registers of the medicines’ usage in farms, in the EU there are legal provisions for it, despite the systematic collection of those records is still not possible to calculate usage data on animals.

Food safety standards [76] have influenced the model of the Portuguese normalised prescription, which is not an EU document, in contrast with the medicated feed prescription (Fig. 3) [21,22].

**Figure 3 - Medicated feed prescription Community model**

A veterinary prescription is “a veterinary act” resulting from a clinical evaluation, and establishing a treatment regimen to an animal or group of animals, while the veterinary requisition serves as a “list” to buy those medicines that are consumed at the farm, on a regular basis; it is also issued to request herd vaccines production [46]. The veterinary prescription for companion animals is not under a normalized model but it is also requires veterinarian validation (Fig. 4).
2.1.1 Veterinary Antimicrobial Medicines Market

In Portugal, veterinary medicines are not commercialized by veterinarians, who are yet entitled to use and dispense it to their patient’s owners [46]. In some EU-MS however, the veterinary antimicrobial medicines are exclusively sold by veterinarians, which may potentially conflict with the professional independency [77], but narrows the antimicrobial retail chain, where the unique supplier is simultaneously the most responsible person on its use and may thereafter simplify the antimicrobial consumption data collection process. On the other hand, precluding the veterinarians from selling veterinary medicines to farmers, it will not necessarily limit an over access to antimicrobial agents, because multiple veterinary prescriptions can be obtained from different veterinarians.

In general and in principle, the pharmaceutical and feed industries, the markets, the farmers, the veterinarians, the slaughter houses and even the consumers would all in principle, from a higher use of antimicrobials in food producing animals, if impacts on public health weren’t so adverse. This has been the rationale to ban the antimicrobial feed additives in the EU-MS, despite being still used for growth promotion in many parts of the world. The animal food industry has indeed a main role in societies and markets, being permanently pressured to produce enough affordable food, with the highest quality possible. From a management point of view, there is a regular tendency to reduce expenses, particularly with veterinary assistance, veterinary medicines and with animal feed that are albeit, all in one, serious microbial resistance risk related factors [78].
2.1.2 Veterinary Antimicrobial Medicines Usage

Administration of antimicrobials to animals always anticipate potential benefits and associated risks. Antimicrobials are indeed powerful tools to control infectious diseases in animals and any reduction of efficacy due to its overuse or misuse is a critical issue in the veterinary sector.

Injudicious use of antimicrobials in animals affects consumer’s health protection from direct or indirect transmission of resistant bacteria (or determinants genes) of animal origin, and increases the probability of occurrence of residues in foodstuff, above the legal MRL established [79]. In order to minimize both consequences, any treatment should use the less possible amount of an antimicrobial and avoid as much as possible the antimicrobials combinations, which are still frequent, particularly in certain oral forms.

The need of metaphylaxis to minimise the health consequences on herds or flocks from severe and highly contagious animal diseases is real, and it may represent a significant level of consumption of veterinary antimicrobial medicines considering that 91.5% of sales for the EU-MS [80] were medicated premixes, powders and water soluble liquids.

The delineation of the epidemiological circumstances under which these treatments are effective, demonstrating a benefit in the treatment of clinically diseased animals, is evaluated under the marketing authorisation procedure with metaphylaxis claim. On the other hand, the claims for the preventive use of the veterinary antimicrobials are only considered in situations where the risk of infection is very high and the consequences are severe. According to the “Good Veterinary Practices”, the responsible use of the veterinary antimicrobial medicines is to preserve their efficacy and efficiency, complying with the information contained in the respective SPC [1,17], after the most appropriated selection of the antimicrobial agent. The options are however mostly influenced by the veterinarians own experiences, the ease of administration to animals, the shortest
withdrawal periods, the medicines prices, and by the results of microbial cultures and sensitivity tests, when indispensable and available [81].

The responsible use of veterinary antimicrobial medicines is shared by the veterinarians with the animal’s owners or keepers (adhering to the veterinary prescriptions or instructions), and also with the NCAs, throughout the marketing authorisations procedure, and over the post-market surveillance, reviewing the granted authorisations, at this stage and whenever a related change in the benefit-risk of the product is evident. Considering that the dosages of the antimicrobials administered may be a key factor either for the efficacy of the medicine or for the bacteria resistance, the veterinary antimicrobials’ quality controls to verify the product stability, are foreseen within the market and under the usual storing conditions at farms. These controls are however scarce at the national level, and at the Community level very few centrally authorised veterinary medicines are tested annually [82,83,84].

The monitoring of the quality parameters, sales and usage of the antimicrobial veterinary medicines can globally contribute to enhance the surveillance of bacterial susceptibility and to sustain the effectiveness of the authorised antimicrobial substances.

In general, antimicrobials should be used where it is indispensable to protect animal health, through the most accurate diagnosis, observing evidence-based treatment guidelines and applying correct dosing regimens and although it may be necessary to use it in the course of specific disease eradication programmes, it should never be intended to replace any biosecurity measures at husbandry.

Currently, the most used antimicrobials in veterinary medicine (penicillins and tetracyclins) are considered to represent a low or limited risk of AMR although macrolides and polymyxins which are classified, at the moment, as first choice antimicrobials for some human treatment are used as well in animals.
3. THE VETERINARY MEDICINES MARKETING AUTHORISATION

Veterinary antimicrobial medicines are products to be administered to animals, containing natural, semi-synthetic or synthetic antimicrobial active substances in its composition, to kill or prevent microorganism’s growth, governed by the Community code relating to veterinary medicinal products. To be granted a marketing authorisation (MA), those products have to comply with the general legal requirements for a veterinary medicine, completed by additionall specific information regarding the microorganism ability to continue to reproduce or to persist in the presence of antimicrobial agents, at therapeutic levels [1,9] defined as AMR.

The veterinary antimicrobials’ marketing authorisation procedure reflects all the relevant information for its efficient and prudent use in the respective labels, leaflets and SPCs [17,85], which are public and available as regulatory tools on the communication of risk management measures, to minimise AMR development [81,86,17]. SPC’s include information [86] regarding the pharmacological properties, target animal species and target microorganisms, indications, dosages, administration routes, incompatibilities, shelf lives, user’s security, precautions, packages and non-used product disposal, withdrawal periods and also risks to public health from the use of critically important antimicrobials (CIAs) in food-producing animals [87].

3.1 Community Regulation

The Community code relating to veterinary medicines establishes the legal provisions for the manufacture, marketing authorisation, supply, prescription and use of the veterinary medicines in the EU-MS, whereas in some other countries only recommendations or guidelines are given to stakeholders.
In the light of the ongoing revision of this EU legal act, it is expected that the benefit-risk assessment for the veterinary antimicrobial medicines may be strengthen and that it may also provide a legal tool to preserve certain antimicrobial agents for human use only, reinforcing the controls of the “cascade” provision [88]. This provision that is used to offset serious lack of available veterinary medicines, mostly in MUMS is a legal derogation to the exclusive use of veterinary medicines in animals or to the use of medicines exclusively under the terms of the MA granted, not preventing any misuse. The “cascade” applies only under a veterinarian´s responsibility, occasionally and to an animal or to a restricted group of animals, any medicine that has been authorised for other target species, for other indications or for human use. It is however fully recognized that the “authorised off-label use” of antimicrobial medicines rarely comply with the undefined frequency and restriction limits, and is therefore particularly difficult to monitor [73] despite the potential impact on food safety, on residue controls and on AMR.

3.1.1 The Availability of Veterinary Medicines

Available and effective veterinary antimicrobial medicines for the treatment of important infectious diseases in animals have to put the minimum risks to both animals and humans. The availability of veterinary medicines strongly depends on markets, that are driven by the type of animal production and animal demographics in each country, [73] favouring major species, as the most consumed animal species in the EU. This problem has however been seriously aggravated by the crescent lack of new authorised veterinary antimicrobial medicines and effective veterinary antimicrobials, due to the increasing emergence of AMR. In addition to the veterinary antimicrobial medicines´ shortage, the categorization of human CIAs [89,90, 91,92,93] that was adopted with restriction use in animals, and the list of specific last resort substances for treatment of life threatening disease in
humans, that should be excluded from animal use, will further contribute to increase the veterinary medicines availability concern. Currently, it is recommended that for low/limited risk veterinary antimicrobials, general principles of responsible use should be applied, whilst those of higher risk, should be exclusively used when no alternative is available for the given species and indication. Further risk profiling is envisaged for certain antimicrobials classes, including those increasingly used against multidrug-resistant bacteria in humans [2] and the life-saving critically important antimicrobials for humans will be excluded from veterinary use. Despite restraints on use of antimicrobial in animals are taken to protect the public health, it is recognized that the resistance to antimicrobial treatments in humans is mostly motivated by the consumption of antimicrobials in human medicine and therefore risk management measures applied to veterinary antimicrobials should be proportionate, and evidence-based only [87].

For the treatment of certain indications in animals, the reformulation of old veterinary antimicrobials with narrow activity spectrum could reduce the off-label use by augmenting the antimicrobials availability, and reduce also the use of CIAs which prescription is often influenced by the shorter withdrawal periods that are usually fixed in the most recent formulations. In parallel, the development of new veterinary antimicrobial agents and of other alternative options such as vaccines and probiotics should be encouraged, whereas the creation of a single market for the veterinary medicines, could be also considered [73] to increase the range of the available products in all EU-MS.

### 3.2 Veterinary Antimicrobial Medicines Marketing Authorisation Dossier

The applications for marketing authorisation of the veterinary medicines are submitted in accordance with the Community code relating to veterinary medicinal products,
supported by the Notice to Applicants and by the adopted Community guidelines that have been developed by the CVMP to assist the quality, safety, efficacy and environmental impact studies.

3.2.1 The Efficacy File

A veterinary antimicrobial medicine is intended to be used only in diseases caused by microorganisms, which are proven or strongly suspected to be susceptible to that active substance, despite the susceptibility for antimicrobials may vary between bacterial species, between strains and over time [86]. Products for MUMS are however subject to specific regulatory provisions [94], in an attempt to increase the veterinary medicines’ availability.

In the marketing authorisation dossier, CVMP and VICH (Veterinary International Cooperation on Harmonisation) guidelines are used to demonstrate the efficacy of the veterinary antimicrobials [86,95,96], which are specific for intramammary formulations and for fixed combinations. Although the misuse of antimicrobial fixed combinations potentially increases the medicine’s toxicity and enhances the selection for resistant organisms, it may be necessary in severe conditions, when a broader activity spectrum is necessary, when a lack of efficacy leads to high morbidity/mortality rates or when the infectious agents involved cannot be identified. Nevertheless, the use of combination therapy may also prevent emergence of resistance, provided that the mechanisms of resistance to distinct antimicrobial agents are different; the chance of a mutant strain being resistant to both antimicrobial agents is much lower than the chance of it being resistant to either one. It provides a better chance that at least one drug will be effective, thereby preventing the resistant mutant population from emerging as the dominant strain and causing therapeutic failure, reason why combination drug therapy is still often used in
veterinary medicine, and because of the limitation of available therapeutic agents as well, whereas in human medicines it is more used when treatment duration is likely to be prolonged and resistance likely to emerge, or due to availability issues too [97].

The efficacy file for the marketing authorisation procedure includes the records of the pre-clinical observations demonstrating the pharmacological action, the mechanisms for the therapeutic effect and the main pharmacokinetic processes [86]. The spectrum of the antimicrobial activity of the substance is defined, and relevant naturally resistant bacterial species are reported.

Veterinary antimicrobials´ indications are established considering where and when the medicine´s use is opportune and when an antimicrobial should be preferred or reserved instead for certain conditions. The effective dose, the dosing interval and the number of administrations of an antimicrobial during the treatment are established in order to last the minimum time possible, taking into account the susceptibility of the target bacteria and the minimum inhibitory concentration (MIC) value, if available.

The MIC is the lowest concentration of an antimicrobial which, under defined *in vitro* conditions, prevents the visible growth of bacteria [86]. Under an adequate dose regimen, the PK/PD relationship gives reasonable confidence whether or not the antimicrobial agent would show clinical efficacy against claimed target pathogens that appear to be susceptible *in vitro* but it is still not well established to predict the optimal duration of treatment. Currently the most commonly used parameters to express it are the Cmax/MIC (maximum concentration in plasma/MIC), the %T > MIC (fraction of time during which the concentration exceeds the MIC) and the AUC/MIC (area under the inhibitory concentration time curve) [86]. Data on the kinetics of bacterial killing characterize the action of the antimicrobial against the target bacteria and demonstrate whether antimicrobial activity is bacteriostatic or bactericidal and whether it is time-dependent,
concentration dependent or co-dependent, in certain diseases [97,98]. The clinical relevance of claimed bactericidal activity against certain target bacteria and mechanisms of the acquired resistance are also evaluated.

For food producing animals and within five years prior to the efficacy file evaluation, epidemiologically unrelated target bacteria are collected, from different farms and from different life-cycles to allow the detection of isolates with MICs deviating from the normal distribution of the wild type strains. If the MIC distribution indicates the presence of bacteria subtypes with reduced susceptibility, these are further compared with historical data to conclude on acquired resistance [86].

Validated MIC test’s data determine the general activity spectrum over a range of microorganisms, which depending on the antimicrobial activity spectrum, may use foodborn pathogenic *Salmonella enterica*, *Campylobacter* spp and commensal *Escherichia coli* e *Enterococcus* spp. Information on resistance mechanisms, on the molecular genetic basis of the resistance, on the occurrence or absence of resistance transmission and the transmission rate, on cross-resistance, including phenotypic and genotypic description, and on co-resistance with another antimicrobials with the same phenotypic and genotypic descriptions, may also be evaluated [99].

When feasible, the epidemiological cut-off value is determined to define the population without any acquired resistance and can be proposed as the clinical breakpoint (i.e. a MIC value under which the selected dose is shown efficient) deviating from the epidemiological cut-off value. The isolates with MICs deviating from the normal distribution for a certain antimicrobial class are further tested for cross-resistance [86,99]. Additional *in vitro* studies may include post-antimicrobial effects and estimation of the rate of selection of resistant mutants, showing how concentrations above the MIC may
affect or prevent mutations. Some environmental factors may be also considered if able to influence the antimicrobial activity in the organism.

Despite the clinical cure rate is generally the primary endpoint of the efficacy assessment, depending on the epidemiology and pathogenicity of the disease, the microbiological cure rate may also be relevant to support the claim. However, on a group/herd level, a relevant efficacy endpoint may be, for instance, just a mortality rate variation [86,95,99].

In the different target species, under the proposed indications and against the target bacteria, the veterinary antimicrobials are tested to be used for treatment, when after the onset of the clinical signs only the affected animals are to be treated; for metaphylaxis, when additionally, there is also a need to administer it to co-habitant animals still clinically healthy but likely to be infected due to the proximity with the disease; and for prevention, when the antimicrobial is administered to healthy animals.

In the veterinary antimicrobial medicines, a metaphylactic claim is only accepted in conjunction with a treatment claim, because some formulations allow a combination of both, as all animals will be treated independently of their individual clinical status. Treatment and metaphylaxis are often mixed as the individual animal health conditions are sometimes difficult or impossible to monitor, but the potential need for metaphylaxis mostly depend on the epidemiology of the disease and on the proportion of the clinically diseased animals in a group.

Privileging the clinical health status of the animals, the efficacy for prevention of clinical disease in unaffected but treated animals is to be achieved, for the best management in farms. Preventive claims refer to administration of antimicrobials to healthy animals to prevent infection, and only when the risk for infection is very high and the consequences are severe [86].
In the efficacy file, the clinical trials serve to demonstrate the effect of the veterinary antimicrobial medicine after administration of the recommended dosage, to guide the usage, to specify indications and contra-indications according to the animal species, age, breed and sex, and to show safety, tolerance under normal use conditions and any adverse reactions. It indicate the harmlessness of the veterinary medicine, producing relevant information on its therapeutic effect, in relations to the indications, contra-indications, dosage, average duration of treatment, interactions with other medicines or with feed additives and any special precautions to be taken during treatment, including clinical symptoms of over dosage. Response to therapy is based on clinical or microbiological criteria for each diagnosed disease.

3.2.2. The Safety and Residues Testing

The safety documentation of a veterinary antimicrobial medicine for marketing authorisation, assesses the potential toxicity of the veterinary medicine in animals and any dangerous or undesirable effects which may occur in animals under the proposed use conditions. The potential harmful effects for humans that may occur during the veterinary medicines’ administration to animals or that may result from exposure to residues (metabolites of the active substances and excipients) present in foodstuffs obtained from treated animals, are also covered [47,48,52].

The evaluation of the toxic effects of a veterinary medicine [48] is based on observations in laboratory animals and target species, under experimental conditions, regarding their behavior and growth, haematology, physiology, necropsy and histological data. The toxicology studies include single-dose and repeated-dose toxicity to predict the possible effects of acute overdosage in the target species or accidental administration in humans, and to reveal any physiological and/or pathological changes induced by repeated
administration, respectively. Reproductive toxicity includes teratogenicity studies to identify possible impairment of reproductive function or harmful effects on progeny and evaluate the effects on reproduction. Any new substance must be assessed for mutagenic properties to assess its potential to cause transmissible changes in the genetic material of cells. For substances to which human beings will be exposed, that have a close chemical analogy with known carcinogens, that inferred possible carcinogenic effects during mutagenicity testing, or that have risen suspected signs during toxicity testing, long term animal carcinogenicity studies are required. When repeated dose studies reveal specific changes in lymphoid organ weights and/or histology and changes in the cellularity of lymphoid tissues, bone marrow or peripheral leukocytes, additional studies of the effects of the product on the immune system may be needed too [62]. The microbiological risk presented by residues of antimicrobial compounds for the human intestinal flora is investigated, and in certain cases, it may be necessary to carry out tests to determine whether residues cause difficulties affecting technological processes in industrial foodstuff processing.

If the constituents of the veterinary medicines are used as human medicines, all the effects observed in humans and on their cause are also assessed. Appropriate measures are always proposed to reduce risks for persons preparing and administering veterinary medicines to animals.

The extent to which food-producing animals contribute to human exposure to antimicrobial resistant microorganisms is difficult to quantify. However, when evaluating the safety of antimicrobial products for use in food-producing animals, regulatory authorities consider the potential for such active substances to select for resistant bacteria [99,100,101] is considered by assessing the characteristics and nature of the resistance, and the potential exposure of target species of the gut flora [100].
According to Bogaard et al., (2000), a major concern is that resistant bacteria, pathogenic or non-pathogenic to humans, are selected in the animal intestinal flora and may transfer their resistance to other bacteria in the human gut [10].

3.2.2.1 Residue Testing

The most relevant safety issue resulting from medicated food producing animals are the residues of the veterinary medicines that may arise in their edible tissues and that may pose a hazard to consumer’s health. Residues of veterinary medicines are covered by specific European legislation to guarantee food security and consumer’s safety, determining MRLs as the acceptable maximum residue concentration in food of animal origin, without any lifetime adverse effect on consumers [1,37,38,48].

If intended to be used in food producing animals, and prior to the submission of the application of a marketing authorisation of a veterinary medicine, pharmacologically active substances, excipients and their residues are evaluated by the CVMP for inclusion as “allowed substance” in the Community legislation [102] (Table 1), indicating which food commodities are referenced for controls. Some substances are evaluated and no MRLs are required on safety grounds while others are prohibited considered to represent a hazard to the safety of the consumer at any concentration in the edible tissues.

Until now, the list of forbidden substances includes Aristolochia.spp and preparations, Cloranphenicol, Clorophorm, Clorpromazina, Colchicin, Dapson, Dimetridazol, Metronidazol, Nitrofurans (including Furazolidona) and Ronidazol, for which was not possible to conclude about its potential toxicity or have been proved to pose a public health hazard, regarding its carcinogenicity, in particular.

MRL determination for veterinary antimicrobial agents are based in the toxicological studies and on resistance development mechanisms in the most sensitive animal species,
by identifying the dosage at which the substance has no observed adverse effects (NOEL- No Observed Effect Level). This parameter is extrapolated to standard human beings (weighting 60Kg) by applying a minimum of a 100 fold safety factor to obtain an acceptable daily intake dose (ADI). This residue dose is then “distributed” by the different constituents of the “food basket” (muscle, liver, fat, milk, eggs and honey), considering the European average food consumption.

The marker residues are those exhibiting higher pharmacotoxicological activity or present in higher concentrations controlled by available validated analytical methods [48,103]. Pharmacokinetic studies are assessed to evaluate the absorption, distribution, biotransformation and excretion of the product in the target species, passage into milk or eggs, the accumulation of lipophilic compounds, and to predict antimicrobial activity in the intestinal tract and frequency in vitro mutation studies with test organisms.

Excretion pathways of the product are described and the major metabolites are identified and characterised.

The depletion of residues studies, determined by appropriate physical, chemical or biological methods, measure the rate at which residues deplete in the target animal after the last administration of the medicinal product, allowing the determination of withdrawal periods [48,85].

Community MRLs are determined to ensure food safety and consumer’s safeguard and are used to determine withdrawal periods (WP), fixed under de MA procedure for each veterinary medicine for food producing animals, reflecting the necessary time for an animal to metabolize the administered product and the necessary time for the product concentration level in the tissues to decrease to a safe, acceptable level (MRL), under practical farming conditions [85].
Table 1 – List of antimicrobial active substances with fixed Community MRLs

<table>
<thead>
<tr>
<th>ACTIVE SUBSTANCE</th>
<th>MARKER RESIDUE</th>
<th>TARGET SPECIES</th>
</tr>
</thead>
<tbody>
<tr>
<td>CLAVULANIC ACID</td>
<td>Clavulanic acid</td>
<td>Bovine and Swine</td>
</tr>
<tr>
<td>OXOLÍNÍC ACID</td>
<td>Oxolinic Acid</td>
<td>All</td>
</tr>
<tr>
<td>AMOXICILLIN</td>
<td>Amoxicillin</td>
<td>All</td>
</tr>
<tr>
<td>AMPICILLIN</td>
<td>Ampicillin</td>
<td>All</td>
</tr>
<tr>
<td>APRAMICIN</td>
<td>Apramicin</td>
<td>Bovine,</td>
</tr>
<tr>
<td>AVILAMICIN</td>
<td>Dicloroisoevermínico</td>
<td>Swine, Poultry, Rabbits</td>
</tr>
<tr>
<td>BACITRACIN</td>
<td>Sum of Bacitracin A, B</td>
<td>Rabbits</td>
</tr>
<tr>
<td></td>
<td>and C</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Not required</td>
<td>Bovine, Ovine (not milk)</td>
</tr>
<tr>
<td>BAQUILOPRIM</td>
<td>Baquiloprim</td>
<td>Bovine, Swine</td>
</tr>
<tr>
<td>BENZYLPENICILLIN</td>
<td>Benzylpenicillin</td>
<td>All</td>
</tr>
<tr>
<td>KANAMICIN</td>
<td>Kanamicin</td>
<td>All (except eggs)</td>
</tr>
<tr>
<td>CEPHACETRIL</td>
<td>Cefacetril</td>
<td>Bovine</td>
</tr>
<tr>
<td>CEFALEXIN</td>
<td>Cefalexina</td>
<td>Bovine</td>
</tr>
<tr>
<td>CEFALÓNÍUM</td>
<td>Cefalónium</td>
<td>Bovine</td>
</tr>
<tr>
<td>CEFAPIRIN</td>
<td>Sum of cefapirina and</td>
<td>Bovine</td>
</tr>
<tr>
<td></td>
<td>Desacetilcefapirina</td>
<td></td>
</tr>
<tr>
<td>CEFAZOLIN</td>
<td>Cefazolin</td>
<td>Bovine, Ovinos, Caprinos</td>
</tr>
<tr>
<td>CEFOPERAZON</td>
<td>Cefoperazon</td>
<td>Bovine</td>
</tr>
<tr>
<td>CEFQUINOME</td>
<td>Cefquinom</td>
<td>Bovine, Swine, Equídeos</td>
</tr>
<tr>
<td>CEFTIOFOUR</td>
<td>Sum of all residues</td>
<td>All mammals</td>
</tr>
<tr>
<td></td>
<td>maintaining the beta-</td>
<td></td>
</tr>
<tr>
<td></td>
<td>lactama structure as</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Desfuroilceftiofur</td>
<td></td>
</tr>
<tr>
<td>CLHORTETRACYCLIN</td>
<td>Sum of active substance</td>
<td>All</td>
</tr>
<tr>
<td></td>
<td>and its 4-epímer</td>
<td></td>
</tr>
<tr>
<td>Antimicrobial</td>
<td>Description</td>
<td>Species</td>
</tr>
<tr>
<td>---------------</td>
<td>-------------</td>
<td>---------</td>
</tr>
<tr>
<td>CLOXACILLIN</td>
<td>Cloxacillin</td>
<td>All</td>
</tr>
<tr>
<td>COLISTIN</td>
<td>Colistin</td>
<td>All</td>
</tr>
<tr>
<td>DANOFLOXACIN</td>
<td>Danofloxacin</td>
<td>All</td>
</tr>
<tr>
<td>DICLOXACILIN</td>
<td>Dicloxacilin</td>
<td>All</td>
</tr>
<tr>
<td>DIFLOXACIN</td>
<td>Difloxacin</td>
<td>All</td>
</tr>
<tr>
<td>DI-HIDROESTREPTOMICIN</td>
<td>Di-hidro-streptomicin</td>
<td>Bovine, Ovine, Goats, Rabbits, Swine</td>
</tr>
<tr>
<td>DOXICICLIN</td>
<td>Doxiciclin</td>
<td>Bovine, Swine Poultry</td>
</tr>
<tr>
<td>ENROFLOXACIN</td>
<td>Sum of Enrofloxacin and Ciprofloxacin</td>
<td>All</td>
</tr>
<tr>
<td>ERITROMYCIN</td>
<td>Eritromicin A</td>
<td>All</td>
</tr>
<tr>
<td>SPECTINOMYCN</td>
<td>Spectinomicin</td>
<td>All</td>
</tr>
<tr>
<td>SPIRAMYCIN</td>
<td>Sum of Espiramicin and Neoespiramicin</td>
<td>Bovine, Chicken</td>
</tr>
<tr>
<td></td>
<td>Spiramicina I</td>
<td>Swine</td>
</tr>
<tr>
<td>STREPTOMYCN</td>
<td>Streptomicin</td>
<td>All ruminants, Swine, Rabbits</td>
</tr>
<tr>
<td>FENOXYMETILPENICILIN</td>
<td>Fenoximetilpenicilina</td>
<td>Swine, Poultry</td>
</tr>
<tr>
<td>FLORFENICOL</td>
<td>Sum of Florfenicol and its metabolites determined as Florfenicola-amina</td>
<td>All</td>
</tr>
<tr>
<td>FLUMEQUIN</td>
<td>Flumequin</td>
<td>All</td>
</tr>
<tr>
<td>GAMITROMYCN</td>
<td>Gamitromicin</td>
<td>Bovine</td>
</tr>
<tr>
<td>GENTAMYCN</td>
<td>Sum of Gentamicin C1, C1a, C2 e C2a</td>
<td>Bovine, Swine</td>
</tr>
<tr>
<td>LASOLACID</td>
<td>Lasolacid A</td>
<td>Poultry</td>
</tr>
<tr>
<td>LINCOMYCN</td>
<td>Lincomicin</td>
<td>All</td>
</tr>
<tr>
<td>MARBOFLOXACIN</td>
<td>Marbofloxacin</td>
<td>Bovine, Swine</td>
</tr>
<tr>
<td>MONENSIN</td>
<td>Monensin A</td>
<td>Bovine</td>
</tr>
<tr>
<td>NAFCILLIN</td>
<td>Nafcillin</td>
<td>All Mammals</td>
</tr>
<tr>
<td>NEOMYCIN (INCLUINDO FRAMICETINA)</td>
<td>Neomicin</td>
<td>All</td>
</tr>
<tr>
<td>NOVOBIOCIN</td>
<td>Novobiocin</td>
<td>Bovine</td>
</tr>
<tr>
<td><strong>OXACYLIN</strong></td>
<td>Oxacillin</td>
<td>All</td>
</tr>
<tr>
<td><strong>OXITETRACYCLIN</strong></td>
<td>Sum of active substance and its 4-epímer</td>
<td>All</td>
</tr>
<tr>
<td><strong>PAROMOMICIN</strong></td>
<td>Paromomicin</td>
<td>All</td>
</tr>
<tr>
<td><strong>PENETAMATE</strong></td>
<td>Benzylpenicillin</td>
<td>All Mammals</td>
</tr>
<tr>
<td><strong>PIRLIMYCIN</strong></td>
<td>Pirlimicin</td>
<td>Bovine</td>
</tr>
<tr>
<td><strong>RIFAXIMIN</strong></td>
<td>Rifaximin</td>
<td>All mammals</td>
</tr>
<tr>
<td><strong>SARAFLOXACIN</strong></td>
<td>Sarafloxacin</td>
<td>Chicken, Salmoníde</td>
</tr>
<tr>
<td><strong>SULPHONAMIDES</strong></td>
<td>Parent drug</td>
<td>All</td>
</tr>
<tr>
<td><strong>TETRACYCLIN</strong></td>
<td>Sum of active substance and its 4-epímer</td>
<td>All</td>
</tr>
<tr>
<td><strong>TIAMULIN</strong></td>
<td>Sum of metabolites that may be hidrolised Para-8-A-Hidroximutulin</td>
<td>Swine, Rabbits, Chicken, Turkey</td>
</tr>
<tr>
<td><strong>TIANPHENICOL</strong></td>
<td>Tianfenicol</td>
<td>All</td>
</tr>
<tr>
<td><strong>TYLMICOSIN</strong></td>
<td>Tilmicosin</td>
<td>All</td>
</tr>
<tr>
<td><strong>TYLOSIN</strong></td>
<td>Tylosin A</td>
<td>All</td>
</tr>
<tr>
<td><strong>TILVASOLIN</strong></td>
<td>Sum of Tilvasolin and 3-O-Acetiltilosina</td>
<td>Swine, Poultry</td>
</tr>
<tr>
<td><strong>TRIMETOPRIM</strong></td>
<td>Trimetoprim</td>
<td>All</td>
</tr>
<tr>
<td><strong>VALNEMULIN</strong></td>
<td>Valnemulin</td>
<td>Swine</td>
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At national level, WP are still often established differently to similar products which have led to some referral procedures, advising further processual harmonization [72].

The residue file presents the extension and duration of residues in the tissues of the treated animal or foodstuffs obtained therefrom and include also practical analytical methods for verify compliance with the withdrawal period routinely. MRLs are reference values for the residue official controls performed to guarantee residues in foodstuff of animal origin below MRLs and as a condition for the international trade of food commodities, among subscriber countries of the World Trade Organization (WTO) under the SPS Agreement (on the Application of Sanitary and Phytosanitary Measures), avoiding discriminations or restrictions [37,38].

3.2.3 The Quality File

The quality file of a veterinary antimicrobial medicine include the qualitative details of all the constituents of the product, quantitative details to specify the mass of each active substance and details relating to the product control tests that may be carried out at an intermediate stage of the manufacturing process, to ensure the consistency of the technical characteristics and the production process. The EMA’s scientific guidelines on the quality of veterinary medicines are specifically provided for non-immunologicals, for MUMS [104] and for post approval change management protocols. It regards in particular, the development of pharmaceuticals, manufacture, active substances, impurities, excipients, packaging, specifications, analytical procedures and analytical validation, transmissible spongiform encephalopathies (TSEs), stability, and specific veterinary dosage forms.

The description of the manufacturing method in the quality file, includes full details on precautions to ensure the homogeneity of the finished product and the actual manufacturing formula. For the starting materials it includes the manufacturing strategy,
the purification/inactivation procedures with their validation and all in-process control procedures designed to ensure the quality, safety and batch to batch consistency of the finished product. Certain tests on the general characteristics of a product relate to the control of average masses and maximum deviations to tests or to organoleptic and physical characteristics. On the stability tests, the manufacturer must propose and justify maximum acceptable tolerance limits in the active substance content of the finished product up to the end of the proposed shelf-life. The recommended storage conditions are described and in the case of the medicated premixes, information is also given on the shelf life of the medicated feed manufactured from these premixes.

Where a finished product requires reconstitution prior to administration, or in case of multi-dose vials, which are very frequent in veterinary medicines, details of the proposed shelf life for the reconstituted product are required and stability is presented to justify a shelf life for the vial after it has been punctured for the first time.

The maximum acceptable level of degradation products at the end of shelf life is indicated and studies on the interaction between product and container are submitted when appropriated.

An in vivo or in vitro biological assay is obligatory when physico-chemical methods cannot provide adequate information on the quality of the product and specificities of safety tests, such as sterility, bacterial endotoxin, pyrogenicity and local tolerance in animals are included in the analytical particulars whenever necessary [48].

**3.2.4 The environmental Assessment Report**

The veterinary medicine’s ecotoxicity assessment is mandatory in applications for marketing authorisation [48] and is conducted in two phases. The first phase assesses the potential extent of exposure of the product, its components and relevant metabolites to
the environment, taking into account the target species, and the proposed pattern of use (mass-medication or individual medication), the method of administration, in particular the likely extent to which the product will enter directly into environmental systems, the possible excretion of the product, its active substances or relevant metabolites into the environment by treated animals, persistence in excreta, and the disposal of unused or waste product. In a second phase, considering the extent of exposure of the product to the environment, and the available information about the physical/chemical, pharmacological and/or toxicological properties of the compound, it is considered whether further specific investigation of the effects of the product on particular ecosystems is necessary. Further investigation may be required on fate and behaviour in soil, water and air, on the effects on aquatic organisms, and on the effects on other non-target organisms, to protect the public, and in particular the workers using such substances and preparations [8,105].

The EMA has published guidance on the environmental risk assessment of veterinary medicines and the regulatory assessment was implemented in 1997 after which, due to an international harmonisation by the VICH, a guidance document on Phase I was implemented in the EU in 2001 [106]. The environmental impact assessment on the potential harmful effects of a veterinary medicines’ use and the necessary precautionary measures to reduce such risks is therefore carried out in two phases, estimating the extent of environmental exposure in the first phase and assessing the fate and effects of the active residues in the second one [62]. L. Bjornerot, (2012) believes that bacterial resistance and the possibly adverse impact of those substances on the environment will be able to be closely monitored in the future [107].

Evaluation of effects in the benefit/risk assessment of veterinary medicines which is mandatory for all new applications and marketing authorisation renewals, includes “any risk of undesirable effects on the environment” and is fully regulated [47,48,108,109].
The risk mitigation measures, on a case-by-case basis, proposed to remove or to restrict the risk associated to an acceptable level, must be in line with the agricultural practice and the mitigation effect should be demonstrable.

As a whole, the environmental risk assessment is structured around the risk quotient approach (RQ) that indicates the likelihood of adverse effects occurring, and is defined as the ratio between the predicted environmental concentration (PEC) and the predicted no-effect concentration (PNEC). The route and quantity by which a veterinary medicine enters the environment determines the type of assessment (Phase I or Phase II) and the scenarios to be used. Dosage, route of application, target species, excretion, route of entry into the environment and agricultural practice, all influence the point at which environmental exposure occurs. The main exposure scenarios relate to the removal of material containing the product (manure, dirty water, fish farm effluent) to, the excretion via faeces and urine (grazing animals) and to the spillage at external application and/or direct exposure outdoors.

The terrestrial environment is mostly exposed via the direct excretion of dung and urine, the loss from animals treated topically, and the spreading of contaminated slurry and/or sludge, while the aquatic environment is exposed via the leaching, the run-off and the drainage from manured land, the direct spillage and/or feed spillage, the direct excretion into water (pasture animals), the direct application in water (like in aquaculture), direct discharge of waste water into surface water, and the release from Sewage Treatment Plants. The active substances can then bound as residues in soil or sediments or be metabolised. Mineralisation or degradation to substances that are part of biochemical pathways are considered as endpoints in biodegradation studies. Often, metabolites of organic compounds are more hydrophilic than the parent compound, and more susceptible
to leaching to groundwater [8]. Exposure of birds, insects [110,111] and mammals is also possible.

The possible development of resistance of natural populations of micro-organisms is not covered by the VICH guidelines [112].
PART II – THE USE OF VETERINARY ANTIMICROBIALS

Antimicrobials have been always used in veterinary medicine to treat and prevent animal diseases and are still used in some countries outside de EU, as growth promoters. Development of AMR has been attributed mostly to human and animal antimicrobial use, estimating equivalent consumptions rates.

For bacterial diseases with complex aetiologies or when treatments do not respond to other approaches, antimicrobials are frequently the only option to the control of subclinical disease and for therapeutic intervention in animals.

All antimicrobials may select spontaneous resistant mutants and resistant bacteria, which can become dominant and spread in host-animal populations. The more an antimicrobial is used, the more likely an increasing number of exposed animals may become resistant populations developing AMR among pathogens and among commensal bacteria, despite the variance of the resistance development rates in the treated individuals, and the coexistence of susceptible remaining bacteria. AMR is a microbiological phenomenon, with or without clinical implications but always a first step for clinical resistance. Increased incidence of resistance development in phytopathogenic bacteria [2,113] has been also reported by Teuber (2004).

The main purpose of an antimicrobial’s use is the rapid eradication of a pathogen causing an infection, with minimal adverse effects for patients, by binding sufficient concentrations to specific vital ‘active sites’ on the bacteria until disrupting its life cycle. The relationship between the concentration and the time at these active sites, (AUC), determining life and death of the bacteria, are unknown and therefore often surrogated by blood AUC. The AUC/MIC parameter represents the degree to which the serum concentration and time exposure of the antimicrobial exceed the minimum needed to
interfere with the bacterial life cycle. As resistance can occur as a result of continuing use of low doses, (infections control/prevention or growth promoters), by selecting microorganisms with higher MIC values, the use of higher AUC/MIC ratios maximizes for bacterial eradication and can minimize the risk of selection of resistant organisms.

The emergence of new diseases for which vaccines are fairly efficient or inexistent, usually associated to secondary bacterial infections, emphasize the role of antimicrobials when used prophylactically or metaphylactically for disease control.

To facilitate herd or flock medication, almost all antimicrobials used for prophylaxis and metaphylaxis are administered to animals via the feed or via the water even though the diseased animals often intake smaller amounts of antimicrobials than healthy animals due to frequent anorexia symptoms associated with infections. Such concentrations, despite being predictably higher than the ‘sub-therapeutic concentrations’ for growth promotion are not at sub-inhibitory concentrations from a microbial perspective, favouring horizontal transfer of genes because at such concentrations, potential receiver strains are not killed. The impact of the withdrawal period on the development of resistant bacteria, remain unknown.

Companion animals which may share common flora with humans are often treated with human medicines.
1. ANIMAL AND HUMAN SECURITY

Antimicrobials with similar mode of actions are widely used to treat and to prevent infection both in humans and animals with remarkable benefits in both cases, except in cases of misdiagnosis, treatment incompliance, or when a medicines quality defect or AMR may occur.

Today there is an overall assumption of an excessive use of antimicrobials both in humans and animals, responsible for increasing the selection pressure on AMR and the veterinary field has been constantly pointed out as a primary source of resistant bacteria, posing high risks to humans, which has been the rationale to ban antimicrobial growth promoters use in the EU, (2006) [114,115], despite insufficient data to support such decision [116], according to the Scientific Committee on Animal Nutrition (SCAN) [117]. In fact, whereas many products used for growth promotion had little or no application in human medicine, products used for prophylaxis and therapy are often close related.

Besides, AMR indistinctly affect the treatment of human and animal’s infections; the transfer of resistant bacteria or of its genetic material between humans and animals occurs, particularly the transmission of resistant zoonotic bacteria via food, and of commensal bacteria (*Echerichia coli* and *Enterococcus*) capable of resistance spread resistance in the host population and resistance transfer to pathogenic bacteria. As an important reservoir of resistance genes, enteric bacteria are the most likely source of contamination of carcasses, and the most important potential link between animal and human AMR [10]. Data demonstrating the magnitude and importance of this transference from contaminated food, water or crops, is however still very limited and was mainly investigated regarding the antimicrobial feed additives.

The potential risk to human health resulting from non-judicious antimicrobial use in food producing animals exist, and can’t be neglected. Some epidemiologic investigations and
molecular subtyping of isolates from human and non-human sources, and despite the heterogeneity of subtypes of enterococci from humans and food-animals, some closely related subtypes often demonstrate an association between use of antimicrobial agents in livestock and AMR among bacteria isolated from humans, supporting the foodborne route of transmission.

There are resistant pathogens in animals but currently only a limited number of important infections have no, or very restricted, treatment options due to AMR and in the Community programmes for AMR surveillance [87], these microorganisms are not monitored to be therapeutically targeted. In fact, the majority of the commensal or zoonotic bacteria, like *Salmonella* or *Campylobacter*, are not associated with serious animals’ diseases. On the other hand, despite the isolation and characterization of commensal bacteria does not distinguish non-pathogenic from pathogenic bacteria, when these are present on herds or flocks, severe nosological impact, often requires mass-medication, and exert further pressure for AMR selection by affecting both types of microorganisms.

Commensal bacteria are frequently present in fresh meat products and may serve as reservoirs for resistance genes that can potentially be transferred to pathogenic organisms in humans. During the animal slaughter and food processing, despite the best practices to prevent it, some resistant bacteria, particularly from gut, like *Salmonella* and *Campylobacter*, may contaminate animal’s carcasses and food which can potentially result in human illness although foodstuff of animal origin is usually thermally processed before consumption, avoiding in principle, such contamination.

The frequency of animal’s treatments depend a lot on the health status of a farm, on the region where it is located and on the animal’s species and genetics within the different productive life-cycles. Foodstuff produced by animals under treatment are rejected over
the treatment period and until the end of the withdrawal periods of the veterinary medicines, after which, residues of veterinary medicines, present in animal’s body or edible tissues, are below the legally fixed MRL. The remaining substances in the animal’s organism as parent compounds or its metabolites, although not representing a safety issue for consumers, may nevertheless still be pharmacologically active, at sub-therapeutic levels which in case of antimicrobials may potentially contribute to amplify AMR pressure. Such effects and others dose related, such as the effects of sub therapeutic concentrations of tetracycline in foetus [118], are however not documented yet. Reporting resistance strains based on phenotype is useful for clinical purposes but it is insufficient to understand resistance, while research on the genotypes of resistant strains may provide a deeper insight into the trend in the evolution of AMR.

With distinct severity, human infections can be acquired from animals also by direct contact and via environmental exposure. In general, the impact on human health is measured by the increase on the frequency of disease, of treatment failures, and on hospitalization and mortality rates. The costs of the treatments are also measured, in both human and veterinary medicine, particularly in food producing animals that are raised with an economic end point but where old efficient and inexpensive molecules (penicillins and tetracyclines) are still mostly used. Some antimicrobials like apramycin, florfenicol, tylosin, tilmicosine and tiamuline have even been developed for veterinary use only, long time ago, being now available as generics and lowering some costs. Since then, few new veterinary antimicrobials have been developed [114]. Some antimicrobial agents recently introduced in human medicine or in the investigation pipeline, are also exclusively reserved for human therapy after [89,90,91,92,93] being classified in accordance with the established criteria of “critically important,” “highly important,” and “important”.

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The link between antimicrobial usage in animals and AMR in humans is hindered by the complexity of transmission routes and ecological aspects of the selection pressure for resistant bacteria. Furthermore, the prudent measures applying to the use of one antimicrobial class may not impact the level of resistance to that class due to cross and co-resistance mechanisms involved, reinforcing the need of a responsible overall reduction of antimicrobials consumption. In the veterinary field and over the last years, a considerable decrease of antimicrobial use has already been possible by promoting animal health \[119\], and improving biosecurity and welfare in farms \[12,73,87\].

The resistance prevalence is likely to increase when antimicrobials are used, and decrease when this use diminishes or discontinues because although individual strains may retain resistance genes, they are often replaced by susceptible strains when the selective pressure is removed \[7\]. However, resistant traits once acquired are hardly removed even in the absence of antimicrobials, despite a greater equilibrium between resistant and susceptible bacterial strains, persisting within different prevalence \[114\]. Some resistance can persist \[2,20\] in hosts for long periods of time without any antibiotic pressure and stabilize with fitness-restoring compensatory mutations allowing resistant strains to compete successfully with susceptible strains in an antibiotic-free environment. Thus, there is no doubt that transfer of resistance genes can occur, but its frequency under natural conditions remain unknown.

To Levy S.B. (2000), the efforts to the return of the susceptible commensal flora is the best allies in reversing AMR \[2\]. Restrictions in antimicrobial use, per se, may not lead to reversibility of AMR in the Community but recently, AMR monitoring became increasingly important due to multiple resistance bacteria emergence \[1,120\] and recurrence of untreatable conditions with current antimicrobials, suggesting impaired
exposure to antimicrobials, to preserve their effectiveness for serious and life-threatening infections.

AMR monitoring is however greatly dependent on the harmonization and standardization of methodologies [1].

1.1 Antimicrobial Resistance

The use of antimicrobial agents in the early fifty’s, has contributed significantly to improve animal health and welfare and to enhance animal productivity as well [1]. Increased use of antimicrobials in humans, animals and crops, soon raised sound concerns related with the emergence of AMR [114], with impact on human and animal health, on food security and on environment pollution [16]. Emergence of AMR has been observed following any introduction of a new class of antimicrobials.

In the seventies and eighties was already very clear that some bacteria had developed or acquired protective mechanisms against antimicrobials to survive but only in the nineties the increasing rate of resistance became more evident.

Antimicrobials administered to animals are essential to prevent the epidemic spread of animal disease and to prevent the transfer of zoonosis from animals to man, although it may contribute to resistance development in animal pathogens and commensals, increasing the risk of human colonization and/or infected by resistant zoonotic bacteria [121]. Indirect effects on human health may also occur, through resistant food-borne bacteria or resistance spread in the ecosystem (e.g., water and soil) although it seems still unlikely that any relationship between antimicrobial use in food producing animals and infections in humans may be fully quantifiable, because the overall risk associated to veterinary usage has always underestimated its potential benefits, by reducing the animal
microbial load and shedding, and producing safer and more affordable food for human consumption.

Resistance may be inherent or acquired [114] and a wide variety of resistance mechanisms can exist for a given compound or class of compounds, without the same clinical relevance or microbiological importance. The complex processes of rapid vertical or slow horizontal spread of AMR is due to the presence of resistance determinants and to the selection pressure which determines the rate and extent of the AMR emergence. The prevalence and persistence result then from complex interaction between antimicrobials, microorganisms, the hosts and the environment [122].

Much of the evidence of the potential transfer of a resistance problem from animals to humans [114,121] regards mostly *Salmonella* [114,123], *Campylobacter*, and some “indicator organisms” (*enterococci* and *Escherichia coli*), which cause no disease in animals but can cause disease in man. The epidemiology of these diseases are complex and include many possible sources other than food animals, and many routes of transmission other than food of animal origin as there are potential sources of resistant enterococci and *Enterobacteriaceae* other than the treated food producing animals. Moreover, it is known that adequate cooking destroys bacteria in food but contamination until ingestion [117] is usually neglected.

Humans and non-food producing animals may also be a source of resistant bacteria for food producing animals, since commensals and pathogens (including resistant strains) can reach the general environment via sewage (animal and human), contaminating also vegetables to be consumed by animals and people. In some countries, according to A. J. Tamhankar (2013) the microbiological quality of water is poor due to the contamination by surface water run-offs, sewage, sludge, animal waste and lack of adequate treatment in the rural areas while in the urban areas it is due to inadequate filtration and treatment,
proliferation of bacteria along de distribution system, inadequate sewage systems, recreational use of surface water, ground water contamination by septic effluent and no treatment of rain waters [19]. Furthermore, antimicrobials are still used to prevent bacterial diseases in plants in some countries.

Without an epidemiological context, demonstration that two genes are identical isn’t enough to spot the source of the infection, route of transmission or carriage dynamics and thus, gene transfer from animal isolates of indicator organisms to intestine isolates in humans, remains unclear.

In general, the resistance patterns of bacterial strains from animals, food and humans are similar, and the assumption that the risk to humans arising from resistant animal enterococci is real but still not clear, because the host’s specificities are expected to play a major role in preventing the resistance phenomenon in the ‘indicator organisms”. On the other hand, the human sewage is also a vehicle of bacteria of faecal origin and may also concur for that risk. Additionally, also *Salmonellae* and *Campylobacter* would be expected to be transferable from humans to animals. It seems however that, for many important human pathogens, antimicrobials use in humans is sufficient to create a major problem and it is believed that the contribution of animals to the AMR issue in humans is inferior.

The importance of the environment as a reservoir for AMR genes is becoming widely, and increasingly questioned, although not properly addressed and considered. The use of antimicrobials in animals, including in aquaculture, leads to the contamination of the environment both with antimicrobials and resistant bacteria, which presence exerts a selective pressure for resistance genes in bacteria in a variety of ecosystems including animals, humans and plants. The use of human antimicrobials medicines have exactly the same impact.
AMR emergence results also from a complex interaction of elements in the environment [19,110,114] (air, soil, and water) with social exchanges (among food producing animals, between animals and farmers, between companion animals and owners), not excluding wild animals, insects and migratory birds), in processing steps (farming activities, transportation, and storage), and in human use patterns (food preparation, meat consumption, and susceptibility to infection) (Fig. 5).

Figure 5 - Interaction of AMR with elements in the physical environment

The cycling of these resistance genes between the different ecosystems is extremely complex and requires further research [16,122] particularly on the contribution of veterinary antimicrobial usage to the environmental resistome.

Different populations of some microorganisms affect animals and man, and the extent to which transfer of resistance genes occurs among these populations is variable. Resistant
bacteria and resistance genes spread from animals to man often spill back into the animal population and are amplified by their movements. The transfer from man to animals via the environment also occurs [121], by various mechanisms Consequently, amplification of bacteria introduced at a low rate to the animal population by faecal–oral recycling, and returned to man via the food chain or other means (direct contact, environmental contamination) is also possible. According to Chris J. Teale (2010), [121] the hypothesis that ESBL-producing E. coli can pass into the environment from humans and that animals both on farms and in the wild may subsequently be exposed to them, has been reinforced.

1.1.1 Regulatory Actions

The Marketing authorisation procedure for new veterinary antimicrobial medicines includes a benefit-risk assessment regarding the acceptable risk to public health and the outcome benefit for the animal health and welfare, in order to reduce the use of critically important antimicrobials and the off-label use, which may constitute a non-assessed risk to public and animal health. The product information of the authorised veterinary antimicrobial medicines recommends responsible use to avoid unnecessary selection pressure for AMR and risk mitigation measures to contain risks for the human health.

In veterinary medicine the need of the off-label use is overall assumed but not duly supported by appropriated treatment guidelines, in accordance to the inherent risks both to animal and human health and to the risk of AMR.

The necessary risk management measures to apply to effective, safe and sustainable new antimicrobials, and on the risk to public health due to the transfer of AMR from animals to humans are however being considered, taking into account the need to protect animal health [87]. For older antimicrobials, it is essential to maintain its effectiveness by monitoring and analysing their sales and usage, monitoring target pathogens and zoonotic
bacteria, and to review the authorisations granted if necessary, particularly regarding any shift on the relation benefit-risk. The development of new and old antimicrobials, especially for MUMS, the development of alternatives, the responsible use of antimicrobials (particularly under the “cascade”), and the recognition that AMR is a global problem affecting both animal and human health is critical. Although there is a real need of effective antimicrobial treatment for relevant indications in all species, the forthcoming Community Regulation on Veterinary Medicines [88] will almost certainly restrict the use of certain antimicrobials in animals to the terms of the granted authorisation and an increased level of innovation on treatment alternatives for infectious diseases, will be urgently needed, including not only vaccines but also immunostimulants, like antimicrobial peptides, bacteriocins, probiotics and bacteriophages, as suggested by Gopal Nath, (2013) [19].

Additionally, the development of new antimicrobial substances, ideally belonging to different pharmacological classes would help to reduce the over reliance on a small number of substances which may accelerate the development of resistance.

Within the post marketing surveillance of the veterinary antimicrobial medicines and based on its risk profiling or on evidence of any change in the AMR risk factors, referral procedures have been already conducted for several products, subject thereafter to re-evaluation of its benefit-risk, eventually determining restrictions on use, indications or target species removal, and strengthening of the responsible use warnings.

As the availability of veterinary medicines is a very sensitive issue in veterinary medicine, and in order to avoid loss of species and indications from older antimicrobial products, the review of the dosage regimens and the subsequent adjustment of withdrawal periods, need to be encouraged [87]. Due to the current differences on the availability of those products, their prices, risk-management measures options, animal production systems and
epidemiological contexts across regions or countries, (including the EU-MS), substantial differences exist in the prescribing behaviours of the veterinary antimicrobial agents, among those countries as well [73,81,121]. Sales differences are likely to be due also to different animal populations, to distinct selection of antimicrobial agents, and to several dosing regimens [124,125]. Antimicrobial class repartition and prescribing patterns vary importantly between species. In the human medicine the factors influencing prescription [126,127] are quite different from those influencing the veterinary prescription but all of them have the common goal to treat and prevent diseases, with the minimum error possible and efficiently.

1.1.2 International Actions

The international cooperation between the World Health Organisation (WHO), the World Organisation for Animal Health (OIE), the Codex Alimentarius and the Transatlantic Task Force on Antimicrobial Resistance (TATFAR) strengthened the fundamental support of a global cross-sectoral approach, in parallel with the national strategies to fight AMR. Several international initiatives have been taken regarding AMR and the use of antimicrobials in animals [1]. Recognising the need of collaboration between human health, animal health and agricultural sectors, the WHO published a draft Global Action Plan [128], outlining the overall public health objectives to ensure continuity of the treatment and prevention of the infectious diseases, with effective safe medicines that are quality-assured, prudently used and accessible to all. Guidance on the risk assessment and responsible use recommendations have been widely produced and there is an ongoing continuous work to ensure that progress made through controlling AMR in Europe, is not put at risk through the “imports of resistance” from
world regions with less rigorous regulation and controls, and where sometimes only recommendations are available and suggested [129]. There is a benefit/risk based approach in the EU for the marketing authorisation of veterinary antimicrobial medicines and the systems for monitoring and surveillance of antimicrobial consumption and AMR are currently being implemented and developed, respectively. However, veterinary antimicrobial consumption worldwide may increase in line with the crescent demand of animal protein and potential higher risks of development of AMR may result from countries with less well developed regulatory systems, potentiated by an increasing international trade and travel [19,130] of both animals and people.

The *Codex Alimentarius* or “Food Code” is about food safety rules to protect consumer’s health and ensure fair and loyal practices in the international food trade. Despite such rules are not mandatory, the *Codex* guidelines and procedures are often endorsed by many countries and often used by the World Trade Organization (WTO) to solve foodstuff commercial disputes. In 2001, the *Codex Alimentarius*, recommended FAO, the WHO and the OIE to consider all issues of non-human antimicrobial use and antimicrobial resistance, establishing a Codex ad hoc Intergovernmental Task Force on Antimicrobial Resistance (TFAMR), [120] to develop science-based guidance to help countries to assess the risks to the human health, associated with the presence and the transmission of antimicrobial resistant microorganisms in food and feed [114], and to develop risk management advice to reduce such risk. The baseline for this Task Force was the risk of AMR increase in humans and animals in consequence of antimicrobial usage in veterinary medicine, plant protection or food processing. The result was «The Proposed Draft Guidelines for Risk Analysis of Foodborne Antimicrobial Resistance» publication, highlighting the need to build capacity to conduct risk assessment and risk management,
and to monitor and surveille the AMR, for continuous routine measurement and analysis of antimicrobial susceptibility testing information to detect trends [131] and for continuous identification of emergence and prevalence of resistant bacteria or genes [132] respectively. Surveillance and monitoring should be conducted, simultaneously in both human and veterinary medicine, sharing data on AMR microorganisms and on risk assessment methodologies, despite there is no harmonized set of antimicrobial agents for resistance testing. As the use of antimicrobial agents is a driving factor for AMR development and spread, antimicrobial consumption figures are consequently required in both sectors [131]. The development of harmonized schemes for monitoring antimicrobial resistance in zoonotic and enteric bacteria (WHO-AGISAR–Advisory Group on Integrated Surveillance of Antimicrobial Resistance) was also particularly recommended [131,133]. As a contribution from the industry (Centre Européen d’Études pour la Santé Animale – CEESA) several microbial cultures collected throughout Europe were susceptibility tested against numerous antimicrobial agents, disclosing a wide range of well-defined zoonotic, commensal and other veterinary organisms for scientific endpoints [132]. Ultimately, the TFAMR concluded on the necessity to continue to fill knowledge gaps with information on antimicrobial usage, surveillance and monitoring of AMR microorganisms, highlighting that the information on the effectiveness of the risk management options, the refinement of the risk communication strategies to ensure transparency and the delivery of accurate and timely messages are essential [134].

Human and animal health professional’s cooperation to adopt all proactive activities that may concur to the prudent use of antimicrobials in animals, assuming no boundaries for AMR, is paramount.
1.2 Food Safety

The use of antimicrobial agents for animal health protection and human protection consequently, may in practice lead to potential food safety issues, and challenge the ability to treat human and animal infections, contributing for AMR selection [120] at the same time. Occurring every day, any part of the world, food-borne diseases are caused by consumption of contaminated foods with a variety of microorganisms. On the other hand, food safety is being enhanced in consequence of more adequate animal health and welfare programmes, decreasing the dissemination and load of some microorganisms [114] and due to the progressive implementation of the HACCP certification within the food industry.

The highest risk factors for the human infections with foodborne resistant pathogens are related to age (particularly the elderly and children), pregnancy, immunocompromised conditions [2,19] (allergic, HIV, oncologic, etc.) and some organic dysfunctions. Diet and exposure of healthy people to high doses of infectious microorganisms are nonetheless, predisposing factors.

Ultimately, the amplification of the AMR phenomenon with emergence of resistant and multi-resistant bacteria, has considerably increased the microbiological hazard to human and animals [120] and the concerns on how to fight it more efficiently.

1.2.1 Foodborne Resistance

Several antimicrobial substances are used to provide healthy animals, high quality crops and to maintain food processing sanitation.

Resistant foodborne pathogens are a subset of foodborne pathogens and do not necessarily cause illness in animals although it may increase the risk of human infection by ingestion, particularly in individuals under antimicrobial therapy and considering that resistant
bacteria may limit treatment options, and resistant foodborne pathogens may therefore develop increased virulence. In all cases, prior exposure of humans to antimicrobials seems to be the greatest risk factor for acquiring an infection with resistant bacteria [135]. Transmission of food-borne pathogens, independently of the susceptibility pattern, has to be in any case minimized at farms, in abattoirs, all over the distribution chain, during food preparation, and even at the consumer’s by promoting the best hygiene practices. Considering the proposed benefits of the antimicrobial medicines in the target species, a certain level of risk may be acceptable on this use providing that it will not significantly compromise the human health. This is certainly a veterinary major concern, although the most representative driver of AMR in people seems to be the use of the antimicrobial substances in human medicine.

Thus, proportionate risk mitigation measures for the veterinary antimicrobial use have to be based on scientific risk assessment, and to consider any potential negative impacts on animal health and welfare. In addition, the effectiveness of risk mitigation measures, applied on a case by case basis, has in most cases to be evaluated in terms of economic impact, assessing also the benefits to animal or public health. [87,136].

The disparity between the trends of the AMR prevalence of certain microorganisms and the trends of the incidence of the antimicrobial resistant infections is still an outstanding issue.
2. ENVIRONMENTAL EFFECTS

Antimicrobial agents can lead to changes in the biodiversity of affected ecosystems, reducing susceptible microorganisms, and developing AMR. The expression of virulence factors and the transfer of antimicrobial resistant bacteria and resistance genes are favored in general by the presence of antimicrobials for a long period of time, at sub-inhibitory concentrations, despite the antimicrobial concentrations found in the environment seem to be not favorable for transfer of resistance and selection of resistant environmental bacteria [16]. Moreover, it is still not clear the frequency, occurrence, extension, faith and effects associated to the presence of antimicrobials in the environment because very little is still known about exposure routes, faith and effects in the ecosystems [16], depending upon the biodegradability and adsorption of the substances, which are related with the drug concentration, stability, persistence in the ecosystems, temperature and other environmental factors.

Many antimicrobials are water soluble and eliminated as metabolites or as parent drugs via urine. Considering that the same drug may be used in several species, to several indications, in different dosages, and over distinct treatment courses, environmental concentrations of those substances vary significantly although always inferior to those administered to animals. The faith in the environment depends on the chemical properties of the parent molecules and its metabolites, on the extension of the biological degradation in feaces, manure, muds or water, on the separation predisposition in soil or water and on environmental characteristics such as temperature and on the type of the soils.

Aminoglycosides and β-lactamases, for instance are readily degradable, tetracyclines and quinolones undergo slow degradation under limited sunlight while macrolides and sulphonamides are least susceptible for degradation [19]. Antimicrobial adsorption rates also vary significantly.
The faith in the environment includes mineralization, biodegradability to carbon dioxide and water, incomplete degradation with mud retention due to lipophilic properties, and metabolism of the lipophilic parent substance to more hydrophilic forms. Some antimicrobials in soil and sludge may even lose its antimicrobial properties after bound to some sludge particles. Mobility in soil by filtration, determines the impact that a substance or its metabolites may have in groundwater and also in terrestrial and aquatic organisms, and although such potential contamination risks are still unknown, crops in contaminated areas with antimicrobials, may carry those substance in its tissues, entering the food chain, with or without animals, in-between the cycle.

In many non-European countries, the environmental aspects related with AMR and the use of veterinary medicines, are still not considered. There are no international codes for the subject despite the important role of the environment in recycling resistant bacteria, within a complex and not entirely well-known system of transmission of resistance among animals, humans, and the environment. Additionally, in some countries the aquaculture systems strongly depend on the heavy use of antimicrobial agents, directly through the aquatic environment where sediments, surface waters, and feed are rich in bacteria. The quality of feed is in any case extremely important. In the EU-MS the feed monitored quality parameters include microorganisms like *Salmonella* but in some regions, even the manure of terrestrial animals is often used to feed fish as a protein source.

The lack of data about the environmental impact from the release of antimicrobial agents used in humans, animals, and plants hinders appropriate risk assessment and management of the impact on health (human and animal), on the environment, and on resultant residues and resistance [16,137].
Nowadays, the environment and the overall related environmental factors are health determinants for the populations. However, the “environmental health” concept still not properly addresses the AMR issues. The predominant role of the human activities in the generation of environmental reservoirs of antibiotic resistance [138] should be further monitored.
3. THE ONE HEALTH PERSPECTIVE

“One Health” is a collaborative effort of multiple disciplines, working locally, nationally, and globally to attain optimal health for people, animals, and for the environment (King et al., 2008). The scale and complexity of the food safety issues request new organizational science team structures, strengthening also in this perspective the AMR issues. Besides some slight inconsistencies on the “One Health” definition, the need of a new framework for preventing food-borne diseases, instead of the usual reactive responses is overall consensual [139]. Prevention and containment of AMR requires a holistic, multifaceted and inter-sectorial approach to face this growing global problem affecting both animal and human health [9].

AMR is a global health problem concurring progressively to more limited or poorer options [19] for the treatment of severe infections and having important economic consequences in healthcare and food production systems. All use of antimicrobial agents, promotes the selection and the dissemination of resistant microorganisms, accelerated by excessive and inappropriate usage and by poor hygiene [114] or poor infection control practices, promoting the emergence of new resistant bacteria through genetic mutations and gene movements.

The innovative incentives for effective antimicrobials, in parallel with the recommendations on the prudent use of antimicrobial agents in human medicine and on patient safety, including the prevention and control of healthcare associated infections clearly show an active regulatory commitment [140,141,142,143] within the AMR issue. To establish the One Health perspective in the EU (Fig. 6), many scientific opinions regarding antimicrobial resistance on zoonotic infections (ECDC, EFSA, EMA and the Scientific Committee on Emerging and Newly Identified Health Risks -SCENIHR) have been taken into account.
In the light of this concept, the EC forward an action plan against the rising threats from AMR (2011) [140,143,144], for which the European Parliament’s Resolutions and the Conference on AMR in Copenhagen, have provided valuable contributions, highlighting the need to avoid overuse of antimicrobials in both humans and animals, with a particular focus on the CIAs policy and on the surveillance strengthen.

Figure 6 - One Health scheme by OIE and EC
All the EU-MS were challenged to implement equivalent national strategies or action plans in both human and animal health sectors, in order to ensure the most prudent use of the antimicrobial medicines and to reduce the AMR inherent risks, including the reduction on the infection transmission rates. The restrictive use of CIAs to cases where no other type of antimicrobials are effective, and a restriction of the antimicrobials prophylactic use to cases with defined clinical needs only should be observed and sufficient microbiological capacity for diagnosis is required.

The available information on the diseases for which veterinary antimicrobials are being prescribed [73,80] is currently limited and very difficult to collect, despite it is considered essential to support guidance on the responsible use of antimicrobials, and particularly regarding the usage of the CIAs in animals. Furthermore, the criteria for prescription and for the sale of antimicrobials in general, should be also carefully examined in the EU, in order to ascertain whether practices in human and animals may lead to over-prescription, overuse or misuse of antimicrobials [81].

In the European veterinary sector, the prescription and the use of antimicrobials in herds/flock treatments, is limited to veterinarian evaluation and confirmation of a clinical or epidemiological justification to treat all the animals, considering that such massive treatments are a main source of AMR contamination.

The AMR eradication is not a realistic goal but surveillance instead, is essential for the strategic containment and mitigation of the problem, providing the access to adequate data that allow locating the AMR issue, and monitoring its development, transmission and direction. Complementary it aids to determine the impact of any taken management measures [9].

A risk based approach holistically grounded on the One Health perspective [145,146] is therefore needed to rationally reduce the use of antimicrobials, maximizing coordinated
efforts between the human and veterinary health sectors against AMR. Definitions of CIAs for humans and for animals by the WHO and by the OIE, respectively, and the restrictive use of such antimicrobials in both sectors with the objective to reserve it as much as possible for human use, have been already an important milestone for the One Health concept.

3.1 Human and Veterinary Antimicrobial Consumption – A Case Study

Humans and animals often consume the same classes of antimicrobials, and despite the more evident AMR problem in the human medicine, animals definitely contribute for it. Moreover, the bacterial virulence is increasing in consequence of infection by resistant strains, often culminating in unexpected deaths, requiring responsible action.

Since early times, and before the recent establishment of the principles for the assignment of the DDDvet (Defined Daily Dose for Animals) and DCDvet (Defined Course Dose for Animals), the comparison between human and animal antimicrobial consumptions was always inevitable and mostly empirically due to the lack of an universal technical unit to measure the veterinary consumption.

Tonnage has been used before to compare human and animal antimicrobial consumptions [147]. In the Portuguese Lisbon and Tagus Valle Region (LVTR), with about 13% of the mainland territory area, the amounts (tons) of antimicrobials consumed by humans and swine, during the same period (2013) were compared. That region is a high density population area (2 458, 4 inhabitants/km) inhabited by 1/3 of the country population (3.7 millions), with 22 human hospitals and a considerable number of pig farms (about 1/3 of the national swine population) (FEPAS), which are not animal health care units despite hosting almost permanently diseased animals under antimicrobial treatment [114, 148].
All the studied farms were licensed to manage their own effluents [149,150] ensuring the legal balance between production, use and treatment, which is out of the scope of the Waste Waters Treatment Units (ETARS) activities. Environmentally, LVTR has some degradation of the hydric resources, intimately related with the existence of many polluting activities of anthropogenic origin, particularly waste water treatment units, collective septic tanks and even direct discharges in agriculture soils.

Emergent biological and chemical threats due to pharmaceutical residues in the environment are subject to sanitarium surveillance, although not covering antimicrobial agents [151,152,153]. The zinc for instance, widely used as an antimicrobial, particularly in the piglet’s diarrheas, is specifically monitored in pig farms for being a heavy metal and to be monitored exclusively as such.

The amounts of the veterinary antimicrobials consumed by swine were calculated from the number of the package units that were sold, using the ESVAC sales data collection model. The medicines used were targeted for pigs and also multispecies presentations (including pigs). No veterinary antimicrobials were identified as having been used under de “cascade” (DGAV). The majority of these veterinary medicines were injectable forms, but oral forms to mix with the drinking water and with feed in farm (topdressing and blending) were also administered. Data on consumption was registered by animal´s different life-cycles (piglets, fattening pigs and sows). The medicated feed consumption was excluded from this study due to data inconsistencies between the medicated premixes sales and its use as medicated feed. Although common antimicrobial classes were consumed by humans and pigs, the consumption profiles were distinct within those classes. In relation to the Lincosamides for instance, whereas clindamycin, fosfomycin and lincomycin, were consumed by humans, only lincomycin was consumed by pigs. Regarding the “other” antimicrobial classes, it covered colistin (Polimixins), florfenicol
(Anphenicols) and tiamulin (Pleuromutilins) in pigs while for humans it consisted of Aminoglicosides, Antituberculosis, Penicillins associations, Monobactams and Sulphonamides.

Data from both human (Infarmed IP) and swine (DGAV) refer to the number of the package units sold by antimicrobial classes and to the tons of the antimicrobials consumed (Figure 7 and 8 respectively). The volumes of the veterinary packages are usually considerably larger, but all the contents were converted to tons due to the lack of equivalent technical units between human and veterinary fields to measure the studied active substances.

![Figure 7](image_url) – Number of antimicrobial packages consumed by humans and swine, in LVTR (2013)

The pharmaceutical forms of the administered antimicrobial medicines were mostly injectable forms in pigs and oral forms in humans, with distinct impact in the commensal bacteria of both populations, considering that the risk of exerting selection pressure for AMR resulting from antimicrobials oral consumption is potentially higher. Because the number of the antimicrobial classes that have been consumed by humans and the range of the available antimicrobial substances within each of those antimicrobial classes were significantly higher than those administered to animals, only the substances commonly consumed by humans and swine that year, were considered for analysis and comparison.
Figure 8 – Amounts (in tons) of antimicrobials consumed by humans and swine, in LVTR (2013)

Four major antimicrobial classes administered to both human and swine have been identified although only the Penicillins were commonly used (17.273 tons in humans and 2.621 tons in swine).

Lincosamids, Pencillins, Tetracyclins and the Pleuromutilines were by this order the most used antimicrobial classes in pigs (Figure 9), while Penicillins, Quinolons, Macrolids and Cephalosorins were by this order the most used antimicrobials classes in humans (Figure 10).

Figure 9 – The four most used antimicrobial classes in swine, in LVTR (2013)
Lincomycin, Amoxicillin, Doxycyclin and Tiamulin, were by this order the most used antimicrobial agents in pigs, while Amoxicillin, Ciprofloxacin, Claritromicin and Cefuroxime were by this order the most used antimicrobial agents in humans. Amoxicillin is recognized to disturb the colonisation resistance of the intestinal tract, facilitating overgrowth and the increasing excretion of resistant bacteria, while tetracyclines are often recommended as first choice antimicrobials in animals [10]. However, the selected veterinary antimicrobials to be used in the different animal species and even for the same indication, vary in the different EU-MS, which may be due to differences related to the veterinary medicines availability at national level, to the authorised administration routes that may differ between countries, to regional patterns of the infectious diseases, to the production systems in place or may simply result from distinct prescribing behaviors [73]. Many old antimicrobials, which are not CIAs, are still the most frequently used and prescribed by veterinarians for food producing animals. Analysis of human prescriptions [126] has also identified differences not only according to the medical specialization and the patient’s age group but also due to the region and year season.
The number of the antimicrobial packages that were sold either to be used in humans or in swine, and the amounts of active substances that were consumed per antimicrobial classes by humans and swine, in LVTR in 2013, may be compared in table 2.

Table 2 – Number of antimicrobial packages sold for human and for swine use and amounts of the antimicrobial classes consumed in LVTR in 2013

<table>
<thead>
<tr>
<th>Common classes s de AB</th>
<th>Human Quantities (ton)</th>
<th>Swine Quantities (ton)</th>
<th>Human packages</th>
<th>Swine packages</th>
</tr>
</thead>
<tbody>
<tr>
<td>AMINOGLICOSIDS</td>
<td>0.00077639</td>
<td>0.4734312</td>
<td>9646</td>
<td>22852</td>
</tr>
<tr>
<td>CEPHALOSPORINS</td>
<td>1.736049</td>
<td>0.07894</td>
<td>249258</td>
<td>11506</td>
</tr>
<tr>
<td>LINCOSAMIDS</td>
<td>0.0004806</td>
<td>7.6606375</td>
<td>394</td>
<td>21371</td>
</tr>
<tr>
<td>MACROLIDS</td>
<td>1.8333126</td>
<td>0.3909</td>
<td>611090</td>
<td>4653</td>
</tr>
<tr>
<td>PENICILLINS</td>
<td>17.27353523</td>
<td>2.62122516</td>
<td>1449892</td>
<td>39907</td>
</tr>
<tr>
<td>QUINOLONS</td>
<td>2.69584265</td>
<td>0.381006</td>
<td>394362</td>
<td>18705</td>
</tr>
<tr>
<td>TETRACYCLINS</td>
<td>0.1464841</td>
<td>1.53655</td>
<td>73438</td>
<td>7187</td>
</tr>
</tbody>
</table>

These figures may be used to compare trends of consumption between similar populations, in similar living and cultural conditions which is clearly not the case; to compare human and animal antimicrobials consumption data, more than an equivalent technical unit of measurement will be necessary, in line with the multiple factors that may influence the analysis and the comparison of both consumption profiles. Despite all the consumption data conversion to tons and the theoretical equivalence of the human and swine biomass in study, human and animal data comparisons should always be very cautious, due to the distinct frequency of treatments along the different animal’s life and life cycles, not comparable to the human’s growth cycles and longevity. These considerations must also be taken into account when comparing antimicrobial...
consumption in humans and animals with appropriate veterinary technical units, equivalent to the human DDDs.

Regarding the amounts of the antimicrobials consumed that year in LVTR, humans consumed more antimicrobials than swine, and critical classes of antimicrobials like quinolones and cephalosporines were also more used in human medicine than in swine production; no epidemiological information was available for evaluation of such consumption profiles.

Actually, the main strategy for both human and veterinary sectors to preserve the antimicrobials efficiency is to reduce antimicrobial consumption, as much as prudently possible. Judicious use doesn’t have however to necessarily decrease to a numeric target but to ensure maximum responsible reduction, instead. Arbitrary goals hardly take all inherent risks into account, particularly those related either with disease outbreaks or with illegal markets.

3.2 Prudent use of antimicrobials in food producing animals

The available guidelines for the prudent use of antimicrobials in animals are essentially similar to the existing guidance for the prudent use in humans.

In food producing animals, the antimicrobials must cure and prevent infections, contributing to improve the overall economic profits of the farms where costs are a ponderable factor [154]. Despite inherent additional expenses, the aetiological or the susceptible microorganisms should always be identified before treatments. Adequate concentrations of the antimicrobial medicines have to be administered accordingly.

There are different profiles of prescription and use of the veterinary antimicrobial medicines in the world [2], including in the EU-MS, but the essential is to ensure that antimicrobial agents are available by veterinary prescription and administered under
veternarian’s supervision only. Eventual incentives deriving from prescription and sale of antimicrobial agents may lead to its inappropriate use and overuse and should therefore be regularly controlled, checking the compliance with the advertisement legal rules on veterinary medicines, which is very complex to control at the farm level.

The microbiological diagnosis and the standard susceptibility tests are main tools for a correct choice of an antimicrobial treatment and an overall de-escalation of empirical treatments [81], contributing this way for the most appropriate use of the antimicrobials, and of the CIAs in particular.

However, the guidance on antimicrobial prudent use must be complemented with harmonized criteria for adequate clinical sampling, diagnostic on-site and susceptibility testing. Educational communication programmes and professional’s training should also be implemented on a regular basis for both human and veterinary sectors.

General prudent use of antimicrobials is upmost recommended [114] ensuring that any usage reduction will harm animals or humans.

3.3 One Health

For an adequate management of AMR, the early detection of pathogenic resistant bacteria in humans, in animals and in food, and the early warning systems for the detection of new resistance mechanisms, have to be part of effective and integrated surveillance systems [120,155].

The international requirements on prescription for antimicrobials, and on surveillance and reporting of antimicrobial use and resistance, defending a global ban on antimicrobial growth promoters (WHO, OIE, and Codex Alimentarius) have been lately intensively promoted. On the other hand, further research and innovation to combat AMR and to
maintain the efficacy and the availability of the existing antimicrobials, should be fostered and supported by specific regulatory measures.

To support the implementation of a comprehensive approach against AMR in the EU, embraced by the ‘One Health’ goal, concrete regulatory initiatives have already been taken (2011). From the EC side, the veterinary working group on AMR was extended to involve the human health issues on the subject, and the Community rules governing either the veterinary medicines or the medicated feed are actually under revision at the Council level. It was also established a network with the EC, the ECDC, ESAC-net, the EARS-net EFSA and the EMA (ESVAC) to strength the assessment and the evaluation of the occurrence of AMR in humans, in animals and in food, despite further work is still required as necessary at national levels through specific measures and actions [140,145,156] to be taken. At least, the visibility and the global awareness of the AMR problematic were raised, developing and strengthening intentions and commitments for its prevention and control that end up to be the milestone of the “One Health” perspective.

The achievements to meet should thereafter rely on effective surveillance systems, in both human and veterinary health sectors, based on appropriate monitoring programmes and allowing the collection of comparable and timely data on AMR and on the use of antimicrobial agents, with the maximum degree of harmonization and procedural standardisation.
PART III – VETERINARY SURVEILLANCE SYSTEMS

The use of antimicrobials in animals is indispensable for the benefit of animal health and welfare but the selective action of the antimicrobial medicines contributes to propagate organisms with resistance genes. Effective surveillance systems are thus essential to understand and to manage the public health risk of the AMR phenomenon, ensuring that the risk management measures to be addressed, are proportionate and based upon robust scientific evidence [2,87].

Adequate risk management to prevent the emergence and spread of resistant bacteria, requires further investigation of the resistance mechanisms [20,114], and of the resistance epidemiology, within the potential resistance reservoirs and multiple resistance links, that are influenced by to many related factors [81] and find in the environment the best conditions to develop and spread.

Food has been always seen as an important source of resistance although close contact with companion animals may also facilitate the human exposure and direct transfer of AMR. According to E. Mateu et al., there are still no data on antimicrobial usage in pets, despite the treatment patterns are today quite similar to the human´s [20].

AMR data in zoonotic and indicator bacteria from food producing animals are currently monitored by EFSA (European Food Safety Authority) [155,157] and surveillance of AMR in animal pathogens has already started in some countries as well.

Due to the crucial role of the antimicrobial consumption data on the AMR management policies, the FAO and the OIE, supported by the EU, have recommended that each country should implement national monitoring and management programmes, orientating results in the best manner possible to effectively contribute to revert the increased tendency of AMR phenomenon, by enforcing the most appropriated measures.
Surveillance of antimicrobial sales in animals have started very recently in the EU and the consumption surveillance is an ongoing project, tested in a pilot phase and temporarily suspended due to some barriers, expected to be overpassed soon.
1. NATIONAL PLAN TO REDUCE ANTIMICROBIAL CONSUMPTION IN ANIMALS

Under the auspices of the One Health perspective, the Portuguese NCA for the veterinary medicines (DGAV), signed up an Alliance for “Preservation of Antibiotics” (2011) with other human and animal health national entities, explicitly indorsing the EPRUMA (European Platform for Responsible Use of Medicines in Animals) recommendations to des-escalate the antimicrobial usage in animals. The EPRUMA is an EU multi-stakeholder platform, linking the best practices for animal and public health with the most responsible use of medicines in animals [158]. Formerly the Responsible Use of medicines in Agriculture (RUMA) had already provided guidance for antimicrobial’s use in livestock [11,121,159].

Additionally, DGAV implemented in 2013 a quinquennial action plan to reduce antimicrobial consumption in animals, grounded on two main strategic areas, defending the sustainability of the existing efficient antimicrobials, and the public health protection by reducing the veterinary selection pressure on AMR. This plan consist fundamentally in the regulatory reinforcement of the inspective measures foreseen in the applicable legislation on veterinary medicines, extended to other public actions that may be pursuit by all the society quadrants, involving in particular the veterinarians and pharmacists, wholesalers and retailers, farmers, consumers, the academia, and the pharmaceutical, animal feed and food industries (http://www.dgv.min-agricultura.pt). Investigation, innovation and technological exchanges to incentive the development of alternatives and alternative measures to the use of antimicrobials in animals to maintain animal health, whenever possible, are also main objectives. Constructive inter-sectorial meetings under the same subject, organized by the veterinary sector, clearly show the importance and the commitment of the animal health for the public health.
2. AMR SURVEILLANCE

The veterinary antimicrobial’s efficiency may be compromised when an antimicrobial concentration is prevented to get to the infection site, when antimicrobials are inactivated by other substances in the organisms or when an organic response is limited. Their efficacy may also fail, in consequence of inappropriate prescription, prescription’s incompliance, or development of resistance by infectious microorganisms. In these cases, the lack of efficacy is obviously not subject to any pharmacovigilance system (human or veterinary) because, although clinical failures may occur, those are not imputable to the antimicrobial medicine itself. Efficacy failures are therefore assessed as programmed reports into the Post-Marketing Authorisation Resistance Surveillance (PMARS) and not as pharmacovigilance unexpected events [24].

AMR monitoring in commensal and food-borne bacteria collected from food producing animals at the slaughter houses is mandatory within the EU-MS, and the veterinary results are published annually [155,157]. The NCAs provide the necessary means to investigate and report the incidence and prevalence of AMR and wherever possible, the epidemiological surveillance of the resistance, which should be complemented with data regarding antimicrobial consumption.

The main objectives of former programmes on pathogenic bacteria surveillance was to give information on AMR levels and guidance to veterinarians and physicians on the treatment of individual cases. For Martel et. al., (2001) the new surveillance systems are however more epidemiology oriented to detect the emergence of new resistance mechanisms, acting like an early warning system, or to follow the clonal spread of resistant bacteria [1].

The need to identify a resistance mechanism, either for monitoring purposes or for infection control, may vary in place or in time, depending on the prevalence and
heterogeneity of the different resistance mechanisms which do not always confer clinical resistance.

2.1 Detection of Resistance
Resistance may be phenotypically or genotypically detected in bacteria.

The phenotypic approach, with the culturing of bacteria and the testing against antimicrobials is the traditional method of resistance detection among microorganisms collected from water or soil, despite the isolation techniques being often highly selective and may therefore miss the majority of bacteria in a sample that are not the study target and the less predominant strains [137].

For clinically important bacteria, phenotypic-based analyses are performed with standardised susceptibility testing methods, usually in accordance with those published by the Clinical and Laboratory Standards Institute (CLSI) [160], as recently recommended by the EU Heads of Medicines Agencies (HMA) [161] for being a method that is based on harmonized veterinary sensitivity testes methodology, and considered to be specific and transparent. In a surveillance programme for veterinary pathogens, it is the use of the antimicrobial agents which is being predominantly analysed, and is therefore accepted that only the CLSI standards offer a sufficiently detailed methodology for it [160] by providing separate standards for human and for veterinary microbiology [162]. CLSI has established antimicrobial susceptibility testing methods for animal [160] and human pathogens, and breakpoints for many microbes and antimicrobials. Currently, there are many different antimicrobial sensitivity monitoring systems being used by the different MS, [120] which are useful to evaluate resistance trends within countries but not for effective comparisons across countries, due to the different factors used to define resistance, particularly regarding the sampling, the sensitivity tests methods, and the
antimicrobials selection. The measurement of the same parameters is not ensured and may lead to misinterpretation of data and inaccuracy of resistance rates comparison [120,131,163]. The interpretation of the Antimicrobial Sensitivity Tests (AST) should consensually recognize the standard meanings [131] of “Sensitive” (S), “Intermedium” (I) or “Resistant” (R) and as wild and non-wild type, under a certain AST procedure, that should also be always identified. On the determination of resistance via phenotype the minimum inhibitory concentration (MIC) can be calculated for each bacterial isolate and antimicrobial drug, and then interpreted as either susceptible, intermediate, or resistant, enabling the most appropriated antimicrobial option for clinical use.

Currently, no standard methods are routinely used in clinical laboratories to determine genotypic resistance and to predict clinical outcomes. Identifying resistance versus susceptibility to some antimicrobial agents may be problematic because there are no standardised testing methods or accepted breakpoint values for many substances. Harmonization regarding the sensitivity to antimicrobials is therefore critical, to interpret and compare available resistance data, which is often obtained from different methodologies and breakpoints in use [164,165]. A universal adoption of an adequate terminology would also contribute to avoid serious confusion when interpreting veterinary therapy guidelines, particularly after emerging changes in bacterial antimicrobial susceptibility, and when it may significate an emerging resistance, demanding appropriate control measures, for public and animal health [166].

For P. Silley (2012), the clinical breakpoint is reserved for the prediction of clinical efficacy, while the epidemiological or wild-type cut-off value is used to separate bacterial populations on the basis of MIC distributions. Clinical breakpoints and epidemiological cut-off values may be very similar or even identical for some bacteria/drug combinations. However, epidemiological cut-off values are determined by a different (and as yet non-
harmonised) approach from that used to determine clinical breakpoints, and do not take into account the results of clinical efficacy studies, dosage and route of administration of the antimicrobial agent, or the drug’s pharmacokinetic and pharmacodynamic parameters in the concerned animal species [166].

The regulatory decisions on responsible use of veterinary antimicrobial agents are usually grounded on risk evaluation models based on antimicrobial sensitivity data, which is not generated or interpreted under harmonized protocols [12,120] and brings thereafter big challenges to the implementation of antimicrobial monitoring programmes and on the use of the epidemiological cut-off values to interpret generated data.

It is therefore essential to obtain a harmonized method to ensure that generated MIC values are comparable too. In order to guide clinical therapy, P. Silley et al. (2012) [131] suggest that antimicrobial susceptibility test data could be interpreted and reported using clinical breakpoints. Conversely, data intended for surveillance purposes should be reported using epidemiological cut-off values, preventing interchangeability. From such remarks it may be concluded that there is a real need to harmonise the agreeing epidemiological cut-off values process.

The European Committee on Antimicrobial Susceptibility Testing – EUCAST, which has established guidelines [167] to detect specific AMR mechanisms of clinical or epidemiological relevance, deals with breakpoints and with technical aspects of the phenotypical STA in vitro, and has already harmonized several breakpoints of antimicrobials MICs in Europe.

The resistance concept, either referring to clinical breakpoints, to epidemiological cut-off values or regarding EUCAST vs. CLSI clinical breakpoints certainly needs clearer definition. To Shabir Simjee (2013) the most important is to ensure that the data being generated is of uniform quality and is interpreted using single interpretive criteria [19].
Overall, surveillance should focus on important microorganisms both to animal and public health and AMR data should be complemented by findings from random sampling in farms, slaughter houses or food establishments, to investigate the resistance prevalence of veterinary pathogens, zoonotic pathogens and alert organisms and to make it available to consult and guidance on responsible use of antimicrobials in animals.

2.2 Resistance Surveillance Systems

AMR surveillance was primarily a tool to monitor local resistance outbreaks or to compare the activity of new antimicrobials against emerging resistances. Several countries have their own surveillance programmes in place, measuring resistance trends over time [168,169,170] but the interpretation and comparisons between country systems are often hampered by a lack of standardised methods, differences in methodology, and a lack of validated interpretive criteria [19]. Antimicrobial monitoring programmes for antimicrobial susceptibilities of zoonotic enteric bacteria isolated either from carcasses or from healthy and diseased humans and animals is essential to better understand the link between human and animal-originated bacterial resistance [132] and to support the risk management interventions guided by risk management.

In food producing animals, *Escherichia coli* and *enterococci* (*Enterococcus faecium* and *Enterococcus faecalis*) are respectively used as Gram-negative and Gram-positive indicator bacteria, due to the high prevalence in faeces of healthy animals and to their ability to harbour several resistances. The purpose of monitoring AMR in indicator bacteria is to avoid overestimating of resistance levels. Susceptibility patterns of indicator bacteria from healthy animals are a good predictor of the resistance situation in the bacterial population.
The European Antimicrobial Resistance Surveillance System (EARSS) is an international network of national surveillance systems, monitoring variations of antimicrobial resistance over time and places with an ongoing surveillance of antimicrobial susceptibility in *Streptococcus pneumoniae*, *S. aureus*, *E. coli*, and *E. faecalis/faecium* causing invasive infections in humans.

An association between the use of antimicrobial agents in food producing animals and AMR among bacteria isolated from humans is most evident among *Salmonella* and *Campylobacter*, but it is also present among enterococci, *Escherichia coli* and other bacteria [132]. Reported data from the EU-MS to EFSA and to ECDC have been analysed in order to monitor the occurrence of AMR in zoonotic bacteria isolated from humans, animals and food in the EU. In 2014, the AMR monitoring of bacteria isolated from food and from food-producing animals [171] was improved, with revised panels of the antimicrobials to be tested and a detailed screening of resistance.

Recently and for the first time jointly, using available data from five EU relevant monitoring networks, the ECDC, the EFSA and the EMA have explored associations between human and food-producing animals´ antimicrobials consumption and AMR in bacteria from humans and food-producing animals [155]. The consumption of several antimicrobials long used in animal husbandry was higher in animals than in humans, while consumption of CIAs for human medicine was higher in humans. Positive associations between consumption of antimicrobials and the corresponding resistance in bacteria were observed in any case, for most of the combinations investigated.

Until very recently, there were few adequate surveillance systems that have yielded information on AMR patterns in clinical isolates of resistant pathogens or gathered reliable data on the usage of antimicrobials and on AMR, though without comparable systems in EU-MS. Efforts are under way to implement integrative surveillance of AMR...
and antimicrobial usage throughout Europe, and at the global level through the WHO’s Advisory Group on Integrated Surveillance of Antimicrobial Resistance. Systems for integrated surveillance of AMR have been established [114] and should generically include systematic sampling, harmonized laboratory methods, and good data management; detailed information on pathogen sample origin, on antimicrobial usage, on sub-typing of bacterial isolates and on molecular characterization of bacterial isolates and of resistance genes are also required. Collaboration on data sharing and comparison should become progressively recurrent [139].

In the meantime, the Conclusions of the Council of the European Union on Antimicrobial Resistance (2008), [141,142] urged the strength of the surveillance systems, improving data quality on AMR and on use of antimicrobial agents within both human and veterinary sectors. Subsequently, the EC requested the EMA to take the lead in the collection of data on sales of veterinary antimicrobial agents in the MS and the ESVAC project was launched in 2009 [120], developing an harmonised approach for data collection and report on the use of antimicrobial agents in animals, based on national sales figures combined with estimations of usage, in major groups of animal species currently addressed in the EFSA reports on occurrence of antimicrobial resistance (EFSA/ECDC, 2012). Comparability with the sale/use of antimicrobials in humans was required and a multi-annual approach should allow the trend´s analysis and evaluation.

The European action plan against the rising threats from antimicrobial resistance (2011), reinforced the importance of the veterinary surveillance systems in veterinary medicine and recommended the extension of the ESVAC project to obtain harmonised data on the antimicrobials usage per animal species and by productive categories. Facing a global challenge, policy makers require standardised data by which will be able to compare different consumption profiles [140,143].
AMR is, for food safety reasons, a public health concern, and surveillance of zoonotic agents is mandatory in the EU-MS [172]. Beside the Community surveillance programmes, some EU-MS have their own national surveillance systems.

The monitoring of *E. coli* and *enterococcus* is only recommended by the EC although it is deliberately monitored by several MS that may determine in their own territory additional microorganisms like *Staphylococcus spp*, *Listeria spp*, and *Streptococcus spp* to be monitored in the margins of the Community official controls.

Such additional surveillance systems are active in France (RESAPATH), Belgium, Denmark, Norway and Finland actually monitoring clinical pathogenic bacteria, whereas indicator bacteria are also monitored by Denmark (*C. difficile*), and by Norway (*Virbrio anguillarum* in fish).

National AMR surveillance programmes on specific pathogenic agents exist for specific animal species, in the Check Republic (monitoring *Listeria spp*, *Staphylococcus spp* in animals including horses), in Germany and Sweden (in all animals), and The United Kingdom in food producing animals.

The Netherlands also monitors several pathogenic bacteria, particularly those associated with most common pathologies like mastitis and with MRSA.

In Portugal, only *Salmonella* and *Campylobacter* are monitored in food stuff of animal origin [157]. Table 3 identifies de internet sites where detailed information can be found on the surveillance systems in the EU countries.

In the EU, Denmark was the pioneer MS on the development of an integrated surveillance system for AMR, throughout systematic sampling and testing of bacterial isolates (pathogens and indicators) collected from humans, food, and food producing animals (DANMAP) and monitoring antimicrobial usage in animals, through the prescribed antimicrobial medicines at a very detailed level (VETSTAT) [173].
### Table 3 – European Network of Antimicrobial Resistance Surveillance

<table>
<thead>
<tr>
<th>EU-MS</th>
<th>Internet site</th>
</tr>
</thead>
<tbody>
<tr>
<td>Check Republic</td>
<td><a href="http://www.vsvcr.cz/">http://www.vsvcr.cz/</a></td>
</tr>
<tr>
<td>Denmark</td>
<td><a href="http://www.danmap.org">http://www.danmap.org</a></td>
</tr>
<tr>
<td>Finland</td>
<td><a href="http://www.evira.fi/portal/en">www.evira.fi/portal/en</a></td>
</tr>
<tr>
<td>France</td>
<td><a href="http://www.anses.fr/index.htm">http://www.anses.fr/index.htm</a></td>
</tr>
<tr>
<td>Germany</td>
<td><a href="http://www.bvl.bund.de">http://www.bvl.bund.de</a></td>
</tr>
<tr>
<td>Lithuania</td>
<td><a href="http://www.nnrvi.lt/en/">http://www.nnrvi.lt/en/</a></td>
</tr>
<tr>
<td>Norway</td>
<td><a href="http://www.vetinst.no/Forskning/Publikasjoner/Norm-Norm-Vet-rapporter">http://www.vetinst.no/Forskning/Publikasjoner/Norm-Norm-Vet-rapporter</a></td>
</tr>
<tr>
<td>Portugal</td>
<td><a href="http://www.efsa.europa.eu">http://www.efsa.europa.eu</a></td>
</tr>
<tr>
<td>Sweden</td>
<td><a href="http://www.sva.se">www.sva.se</a></td>
</tr>
<tr>
<td>The Netherlands</td>
<td><a href="http://www.cvwur.nrl/UK/publications/otherpublications/maran/">http://www.cvwur.nrl/UK/publications/otherpublications/maran/</a></td>
</tr>
<tr>
<td>United Kingdom</td>
<td><a href="http://vla.defra.gov.uk/">http://vla.defra.gov.uk/</a></td>
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<td><a href="http://www.defra.gov.uk/ahyla/">http://www.defra.gov.uk/ahyla/</a></td>
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<td></td>
<td><a href="http://www.afbini.gov.uk/">http://www.afbini.gov.uk/</a></td>
</tr>
</tbody>
</table>

This system supports the “Danish yellow card” system, which identifies high-usage producers, warns their veterinarians, and encourages reduction in usage, under official supervision.

The Danish project to reduce antimicrobial’s use in animals (mostly in pigs) was supported by other important measures, including in particular the selection of appropriated genetic lines to breed more resistant animals and the political strength of the productive sectors to stimulate competition in the international markets.

Despite the widespread use of antimicrobials in food producing animals, reliable data on quantity and use patterns (e.g., dose and frequency) are very recent. Quantifying antimicrobials use in food animals is challenging, because although limited, the available data suggest that animal production is responsible for a significant proportion of antimicrobial use.

Complex political, economic, and social barriers have limited the quality of data on the use of antimicrobials in food producing animals [12,154], which is currently provided on a voluntary basis. Data on veterinary antimicrobial consumption to better characterize the
risks to human health and the benefits to animal production may become economically important, [136] due to the expanding global trade of animals and animal foodstuff and to the increasing restrictions related to antimicrobial usage, conditioning the trade (imports and exports).
3. CONSUMPTION SURVEILLANCE SYSTEMS OF ANTIMICROBIAL AGENTS - THE ESVAC PROJECT

Data on the usage of antimicrobial agents in animals is essential to identify and quantify the risk of developing and spreading AMR, particularly in the food-chain, among key foodborne bacteria and clinical pathogens [174].

To report antimicrobial consumption in animals different units of measurement have been used besides the kilogram, but even the most identical units were defined and calculated differently in each MS.

For V.F. Jensen et al. (2004), [163,175] measuring veterinary usage by the weight of active substances consumed, has clear limitations in analysing the impact on development on resistance, as the unit of measurement has to take into account the potency and the formulation of the antimicrobial medicines to evaluate the therapeutic effect and the selective pressure in consumption studies.

In the absence of consumption data of veterinary antimicrobial medicines, sales data may be used to assess the exposure of animals to antimicrobials, which was the baseline concept for the development of the data collection model on veterinary antimicrobials usage by animal species, by the ESVAC project.

Currently ESVAC has three work streams ongoing: the collection of overall sales data, the development of data collection systems on consumption by animal species and the establishment of technical units of measurement. The collection of harmonized data and report of standardised data on consumption per species and, when applicable, on animal population ‘at risk of treatment’ was tested in a trial conducted in 2014-2015 in twenty pig farms of ten Member States (MSs) but its revision is still needed. Insufficient level of support was given to this pilot study mainly due to the lack of a specific legal basis to develop it at MS level but also because of the complexity of the planning process,
duplication of regulatory activities and lack of human and funding resources [18]. Although at a long term perspective, a sustainable approach, will rely on delivery records (automated or semi-automated data collection) or on the collection of electronic prescriptions [176], covering all farms or a representative number of farms. Such representative number should be revised, as the ESVAC “number” of the farms tested in the pilot phase is very much discussable.

The EU legal code for the veterinary medicines establishes that veterinarians and animal owners must keep detailed records of the veterinary medicines. These should be registered for each use, by date, name, administered dose, name and address of the supplier, and identification of the treated animals [48,177] individually and groups. However, the ongoing revision proposal [88] which is expected to come into force in 2019, should expressly foresee such detail, already mandatory in Portugal, since 2009.

The current available data already allow an integrated analysis with data on use of antimicrobials in humans, and on resistance in animals, humans and food [178], as part of the Joint Interagency Antimicrobial Consumption and Resistance Analysis Report (JIACRA).

3.1 Sales Monitoring

The EMA has been collecting data on sales of veterinary antimicrobial agents from EU-MS and EEA countries under the ESVAC project [124] since 2009. Compared sales are considered to be essential [131] to identify and quantify risk factors for the development and occurrence of resistance in animals [179] as for their impact on human health.

Initially, only nine EU/EEA countries with available official data on sales of veterinary antimicrobials, (though distinctly reported), have participated in the ESVAC pilot study on sales, providing a harmonised collection and standardised report of aggregated data.
regarding 2005-2009 [124]. The trends in sales over time and the trends of the prescribing patterns within those countries, were analysed for the first time. Sales data continued to be voluntary ceded to EMA by 19 countries in 2010 [180], followed by 22 countries in 2011 [181] and by 26 countries in 2012 and 2013 [80,182], covering approximately 95% of the food-producing animal population in the EU/EEA.

3.1.1 Sales Monitoring Model

Data on sales of veterinary antimicrobial agents have been collected from the distinct nationals’ marketing channels (wholesalers, MAHs, feed mills, pharmaceutical industry and pharmacies) under an established protocol and a data collection model, at package level and subdivided by different classes and formulations. Totals are calculated giving an overview of the overall sales by veterinary antimicrobial pharmacological classes in the MSs, allowing an analysis of the sales trends but providing no information on the consumption patterns in animals by species, weight groups or production type. Annual sales are related with the animal populations in each country, which may vary over time, and are divided by the estimated animal weight at farm and after slaughter, using the population correction unit (PCU) as a technical unit of measurement for that estimated weight, used only to estimate temporal trends in countries and across countries. It was assumed that 1 PCU = 1 kg of food producing animals and slaughtered animals. Cats and dogs are still not included in the PCU, expressly created for the ESVAC. Regarding food producing animals, the Eurostat, (the Statistical Office of the European Union), is the data source of numbers and of biomass of the slaughtered animals while national statistics are consulted for horses. The number of animals exported or imported, for fattening or slaughter (likely to have been treated in the country of origin) is obtained
from TRACES (Trade Control and Expert System) database, grounded on health certificates, which are mandatory for trade.

However, sales in mg per PCU is not an indicator of the animal’s level of exposure. It serves instead to adjust trends in the sales within a country and to relate possible changes, like the livestock population and the number of the slaughtered animals.

The ESVAC template for the sales data collection, by aggregated data, provides detailed information at package level on the amounts of active substances and administration forms.

3.2 Antimicrobials Consumption Monitoring

For adequate and effective policies against AMR, reliable data on antimicrobial consumption, is necessary for suitable profiling, assessment and communication of the AMR inherent risks, and for setting risk-management priorities, monitoring the effectiveness of implemented control measures. Integrating data on usage of veterinary antimicrobials with AMR surveillance data, it will help to identify emerging uses, and to interpret the patterns and trends regarding AMR. Furthermore When segregated by animal species, it can be used to estimate the number of animals exposed to a specific antimicrobial class within a given time period.

For the best guidance on antimicrobial´s responsible use and sustainability, representative, accurate, reproducible and detailed data on antimicrobial consumption by species, is required. In order to standardise reporting and facilitating the objective interpretation of results, adequate technical unit(s) of measurement have to be used in an integrated analysis of resistance in animals and humans. This unit has to be suitable for describing and comparing antimicrobial consumption in a wide variety of treatment settings in different populations and over time (V.F. Jensen et al., 2004) [163,175]. The
ESVAC project has recently started the overall data collection on consumption of veterinary antimicrobial agents by animal species. Data sources and data collection methods vary between countries. These should be feasible and sustainable, ensuring comparable data with a minimum level of precision and reproducibility. The applicability of the ESVAC model is particularly useful for the repartition of the total sales, based on unit(s) of measurement [183,184] that have to be established to properly quantify the amounts of the antimicrobials sold and consumed by animal species.

The antimicrobials consumption data, either to be used in risk assessment or for integration with antimicrobial resistance data, has to be accessed by active substance, because the selection pressure to be evaluated is related to the use of a specific antimicrobial agent or class, even when administered in fixed combination medicines. In relation to the long acting products, usually administered by parenteral route (which are becoming increasingly frequent), the duration of the action is taken into account in the assignment of the veterinary technical units of antimicrobial measurement.

### 3.2.1 Data Collection by Animal Species

Consumption data by animal species, is supposed to allow the analysis of trends in use of antimicrobials over time, and to enable comparisons across countries. Ideally, to get the most accurate estimates, data should be collected continuously from farmers’ or veterinarians’ records or using a suitable observational study design instead. The cross sectional type [185] is based on a single data collection event, and gives retrospective information for a given time period from the farm’s records on the veterinary medicines administrations, to calculate the amount of the antimicrobial agents used by species and per life cycles, with different weight groups.
The number of the involved farms need to be representative to allow extrapolation of results to express the nationals’ estimates of the annual consumption per animal species. Alternatively, prospective longitudinal studies may be used from monitored farms over a certain time period, during which all treatments are recorded in predesigned sheets or in electronic data recording systems either by the farmer or by the veterinarian. This model may have the advantage of collecting data in a more complete and standardised manner, avoiding the problem of recall bias. Consumption data may also be recalled from veterinary prescriptions or farm’s delivery databases if provided with sufficient detailed information to assign the products to a specific species and weight group. The overall accuracy of this data strongly depends on the availability of the farms documentation of such treatments.

Data on prescribed or administered antimicrobial agents (name, number of packages, form, strength, dosage, treatment duration, and number of treated animals) is usually obtained from farmers, veterinarians, pharmacies and feed mills to calculate the consumption amounts. Farm data is used to calculate the population at risk of being treated, concerning the type and the numbers of the animals at the farms, including the length of the production cycles. However, data may be obtained from national databases and eventually completed by the farmer, or fully given by the farmer.

To calculate the antimicrobials consumption within a population at risk for treatment, the information regarding the veterinary medicines (name, form and strength, number of packages, animal weight group), the treatments that were administered to the animals in a herd or flock (name, form and strength, daily dosage and treatment duration), and the number of the treated animals segregated per body weight, are collected for a defined period of time. The value of the data collected from the sampled farms, (even when randomized) are significantly dependent upon the farmer’s cooperation, and the
extrapolation thereafter to the entire national population requiring therefore particular caution and capacity to demonstrate de utility of results.

The average weights at treatment (ESVAC, 2013) varies considerably, within the different life-cycles of the different animal species and standard weight classes were already assigned, as a compromise, for the various animal categories.
4. TECHNICAL UNITS OF MEASUREMENT AND INDICATORS FOR CONSUMPTION REPORT

In human medicine the DDD is the standard technical unit of measurement of antimicrobials consumption, representing the assumed average maintenance dose per day for a medicine used for its main indication in adults. It is assigned at international level (WHO Collaborating Centre for Drug Statistics Methodology, 2011) and revised whenever necessary or at least, three years after marketing [131] for standardised report of the consumption of antimicrobial agents by classes, time periods and countries. The most used indicator to present the consumption of antimicrobial agents is the number of DDDs per inhabitant per day (DDDs/1000 inhabitants/day), providing a rough estimate of the proportion of the population treated daily with a particular antimicrobial agent or class, and not taking into account any differences in the treatment duration of the various antimicrobial agents.

Similar standard units of measurement for veterinary antimicrobials consumption (DDDvet/DCDvet) are also essential despite unsuccessful attempts in a recent past. The DDDvet and DCDvet assignment is consigned to the EMA at the ESVAC level, which is composed by the EU-MS and where the OIE, as the homologue of the WHO, detains exclusively the observer status.

A technical unit to report consumption of veterinary antimicrobial agents aggregated by classes for each animal species and weight group summarises the consumption of different antimicrobial agents with different daily doses and treatment duration. This also allows comparison of consumption between species and weight groups, route of administration and antimicrobial classes across time and countries [186] and facilitates the analysis of the data together with consumption data from the human sector.
Furthermore, the adoption of these units allows harmonization of the reporting of the ESVAC data with that from human medicine (ESAC-Net).

The data required by ESVAC for assignment of the DDDvet (and of the alternative unit DCDvet), per animal species, refers to the countries’ estimated amounts of each antimicrobial agent used per weight group/production type. The animal population data at national level, is suggested to be obtained from the same data sources that are already used to monitor veterinary antimicrobials sales.

Regarding the food producing animals, the animal population in risk of being treated is obtained or refined at the farm level, and from Eurostat and TRACES when any found deviation in the national statistics is more than 5%.

Regarding the companion animals, other than horses, which have dual quality under de EU legislation [40], the accuracy of the national registers will be more difficult to obtain but consumption can nevertheless be measured through the electronic prescription together with information collected at the animal health care units.

The DDDvet, similar to DDD, is usually based on the SPC’s recommendations and it is assigned per kg of treated animal in a given species, for its main indication, taking into account the differences in the daily dose for the individual active substances. The DCDvet, is an additional unit that can be used to take into account the daily dose and also differences in treatment duration. Both units are assigned by kg bodyweight and by animal species [18].

In practice, the technical units of measurement are the amount of the antimicrobials consumed, whereas indicators refers to the number of technical units of measurements consumed normalized by the animal population at risk of being treated in a defined period. To allow comparison between periods and countries, data have to be reported together with the population at risk of treatment. This allows correction for changes in the
population according to time and permits also to estimate the proportion of the subpopulation treated in comparison to the total animal population at risk of being treated. For the ESVAC project, data regarding each antimicrobial agent must be provided as the real or estimated consumption, in tons, by species and by weight groups, to be analysed and reported by DDDvet and by DCDvet units with the appropriate denominator. The indicators used by country to report the consumption data per 1000 animals by species (per weight group/production type, per year), are the weight of the active substance consumed in mg or the number of DDDvet/ DCDvet consumed.

Data collected from a representative number of farms have thus to be extrapolated in order to provide estimates on consumption within species and weight groups, requiring in time information on the total number of animals produced for the year in question.

The resource-demanding nature of the ESVAC project has to be compatible with accuracy as all national results will be published and used worldwide.

Integrated surveillance systems have always to evolve to face new challenges but it is also crucial to be compatible with others already existent [120].

Further refinement of the ESVAC data collection on antimicrobial consumption by species, coupled with improved surveillance of AMR, including target pathogens, will allow a better focus on AMR risk management measures.
THE THESIS OBJECTIVE

Considering the role of the veterinary medicines in the public health and in the economy, inevitably contributing to the AMR growing threat and the major importance of a responsible intervention on the overall containment, supported also by efficient and reliable surveillance systems on veterinary antimicrobial’s consumption, the main objectives of this thesis are to:

- contribute for a better understanding of the specificities regarding the veterinary antimicrobial medicines and the medicated feed in the regulatory context, requiring further harmonization on concepts, legal interpretations, and actions to respond to the EU continuous challenges;
- highlight the primary characteristics of the responsible use of antimicrobial substances in food producing animals, and the need to rationally adapt the European strategy against AMR, to the collection and report of available accurate data on antimicrobial sales and consumption, with benefit for health and without prejudice of the countries’ economy;
- support the best performance of the ESVAC developments and achievements, particularly regarding the further need of refinement of the project and the collection of reliable data on use of antimicrobials by animal species.
- share how much has been done in the veterinary sector to produce safety food and how much this sector is willing to continue to protect animals and people.

This thesis is introduced by a personal view about the impressive use of the human knowledge in the most advanced technologies that have changed people’s lives and mentalities in many parts of the world over the last 50 years. Suddenly, the economy became an important driver for the highest quality life standards and influencing food
behaviors. The food industry increased significantly and nowadays it is probably the most powerful sector in the world in parallel with the pharmaceutical industry. Animal production for human consumption is strongly related to both sectors highlighting the relevant role of the regulatory intervention to protect health and to guarantee security [187]. Surprisingly, the phenomenon of AMR has aggravated [188] to a point where the only containment solution seems to be the de-escalation of antimicrobials consumption in a brand new societal format involving the human sector with a One Health Only perspective, despite the many doubts around the problem still to ascertain, including the impact of the de-escalation of antimicrobial use on the AMR. Nevertheless, lack of data allow speculations whereas available data may give proper answerers and it is time for the veterinary and human sector to disclose information to jointly tackle the AMR issue, developing integrated surveillance systems. The “Introduction” of this thesis is divided into III Parts. Part I presents a short synopsis of the legal history of the veterinary medicines in Portugal, and describes the regulatory scope of the veterinary medicines, including the requirements for the marketing authorisation procedure and the marketing specificities at the national level. Part II, on veterinary antimicrobial’s use, describes its role when administered to animals and mostly to food producing animals, linking this usage to the human safety, in the light of the AMR threat and highlighting the concerns and the actions that have been, or have to be taken on this regard, assuming the One Health perspective and the environment. Part III, recognizes the importance and need of surveillance systems in veterinary medicine, as recommended in the EU, to adequately monitor antimicrobial consumption in animals by species and resistant bacteria in integrated programmes. It also describes
the ESVAC project, as the most advanced and harmonized step towards surveillance in animals in parallel with the human sector, caring also for similar and comparable measurement approaches.

After this introduction, the thesis is compiled in VI Chapters.

Chapter I – ANTIMICROBIAL’S SALES MONITORING MODELS, Sales of antimicrobials for veterinary use in Portugal between 2006 and 2009;

Chapter II – ANTIMICROBIAL CONSUMPTION BY ANIMAL SPECIES, Antimicrobial consumption in swine in Portugal: estimation of the population factor to monitor antimicrobial resistance;

Chapter III - FACTORS INFLUENCING ANTIMICROBIAL CONSUMPTION DATA COLLECTION, Trends in the consumption of veterinary antimicrobial agents in pigs;

Chapter IV - THE QUALITY OF THE VETERINARY ANTIMICROBIAL MEDICINES, Oral Veterinary Antibiotic Dosage and Antimicrobial Resistance Promotion.

Chapter V - Discussion and Chapter VI is the thesis conclusion.

Published articles and articles to be submitted are part of the Annex.
CHAPTER I

ANTIMICROBIAL’S SALES MONITORING MODELS

Sales of Antibiotics for Veterinary Use in Portugal Between 2006 and 2009

Abstract

Antimicrobial resistance has been recognized worldwide as a serious public health threat that has spread in global dimensions. Currently, epidemiological studies have shown a consistent and statistically relevant relationship between the consumption levels of specific pharmacological classes of antibiotic and resistance to those drugs. As an effective strategy for antimicrobial resistance contention, the rational use of these medicines in both human and veterinary medicine as well as the prevention of resistant strains in the context of the “One Health” concept require data on antimicrobial sales and consumption. Data regarding antimicrobial sales in Portugal between 2006 and 2009 were collected following two distinct models. The first model, implemented from 2006 to 2007, was developed by the Portuguese General Directorate of Food and Veterinary Affairs for national monitoring purposes in the framework of a Technical Commission for Antimicrobial Resistance Prevention. The second model aggregated data between 2008 and 2009, retrospectively, according to the current model, which was developed by the European Surveillance of Veterinary Antimicrobial Consumption in 2010. Based on data collection of veterinary antibiotic sales, this paper will increase the knowledge about the link between antimicrobial sales and the occurrence of resistant strains, by presenting, for the first time, data from Portugal and by comparing data obtained by two different models.
1. Introduction

Antimicrobial veterinary medicines usage is essential for treating and preventing contagious diseases among animals including zoonosis [189,190]. After treatment and an appropriate withdrawal period, healthy food-producing animals can produce food for human consumption. Food demand, cost, and quality, are angles of a triangle, and the shape of this triangle may vary according animal production practices [191]. The “One Health” claim should be kept in mind because “Antimicrobial resistance is, at the present time, one of the major public health threats” and epidemiological studies have shown a consistent and statistically relevant relationship between consumption levels of specific antibiotic pharmacological classes and resistance to those classes [15]. The transfer of resistant bacteria is not restricted to a particular country or to several countries although they might share common regulations such as the EU. Trade of animals and animal food products and dissemination of bacteria with transferable resistance genes is a world-wide issue [7,192]. In 2008, the European Council called upon the EU Member States to strengthen their surveillance systems on AMR (antimicrobial resistance) and on the use of antimicrobial agents in the veterinary sector. Consequently, the European Commission requested that the European Medicines Agency (EMA) coordinate the collection of sales data by implementing a new and standardised approach of data collection and broadcast. This approach was based on the sales figures of each MS (member state) combined with the use estimation for major groups of animal species, such as poultry, swine, ruminants, pets and fish, and comparing these data with the obtained from surveillance of human antimicrobial use [180]. This project, later called ESVAC (European Surveillance of Veterinary Antimicrobial Consumption), was launched in 2009 to develop an approach for the standardised collection and data reporting on the use of antimicrobial agents in animals in the Member States of EEA (European Economic Area). This European
network began collecting data in 2010 and 2011, but not all countries have actively participated. Thus, one of the most feasible strategies for the contention of microbial resistance is to use antibiotics rationally and prudently and to avoid the spread and transmission of resistant bacterial strains [193,194]. According to ECDC (European Centre for Disease Prevention and Control) data, Portugal has one of the highest rates of antibiotic resistance in Europe [29], and the Portuguese authorities, both human and veterinary, are well aware of detected and analysed transferable antibiotic resistance and that reservoirs of antibiotic resistance genes in bacterial flora of different ecosystems communicate [7,193] therefore committed to reviewing this score quickly and efficiently [189,194].

For this purpose, it is fully recognized that only reliable data may indicate the best course of action for the contention and management of microbial resistance [180,195]. Once collected, data on antibiotics usage can be valuable indicators for policy makers to decide strategies on resistance control, both nationally and abroad and may contribute to knowledge of the potential for overall development of antimicrobial resistance in the veterinary field [196].

The use of human antimicrobial medicines in animals must be considered, although this use is most likely greater in companion animals rather than in food-producing animals due to the marked disadvantage of the quantity versus price relationship. However, these sales are monitored as human antibiotic sales despite its eventual use in animals [197].

The collection of veterinary antibiotic sales data in Portugal began in 2006. However, such data collection was performed using a model developed by DGAV (Portuguese General Directorate of Food and Veterinary Affairs) for national purposes. In 2010 the ESVAC developed a new model for collecting this data [180]. This new model was retroactively applied to monitor veterinary antibiotic sales in Portugal between 2008 and
Thus, the main goal of this study is to increase the knowledge of veterinary antibiotic sales by presenting, for the first time, data from Portugal and by comparing the data obtained by the two different models.

2. Materials and Methods

2.1. DGAV Model

The Technical Commission for Antimicrobial Resistance Prevention was assembled in 2008 by mandate of the Portuguese Ministry of Health to conceive, implement, monitor and evaluate a national programme on AMR prevention [15]. The model for the collection of data on sales of veterinary antibiotic medicines between 2006 and 2007 was created at DGAV in late 2008. Veterinary prescriptions had recently become an official document for food producing animals that had to be validated by an official personalized veterinary stamp, and a registry of all medicines administered at the farm level was also created, first in an official book and later in a regular book or informatics format as suitable [45,46]. However, for daily needs, veterinarians may list in a veterinary requisition the medicines they wish to dispense indoors, which must be also validated. Requisitions may be directly supplied by wholesalers, but either human or veterinary prescriptions must be distributed by pharmacies.

Despite the mandated 5-year period of record keeping [45,46] by veterinarians, farmers and pharmacists, these documents were not an appropriate source of data for monitoring veterinary antibiotics sales because veterinarians may purchase, transport and dispense veterinary medicines as part of their practice to livestock that are in a different location from where the prescriptions were first registered. The system used to document
medicinal use in livestock and to control acquisitions legality, storage conditions and registers compliance of the presence and use of medicines and veterinary medicines, which compares prescriptions; requisitions copies; and wholesalers’, pharmacies’ and veterinarians’ receipts with administration registers, parallels the nationwide execution of the Portuguese National Residue Control Plan by DGAV within a significant number of inspected holdings. However, this system does not enable local data collection from registers in a reasonable and adequate manner.

In 2009, a legal obligation aiming AMR, reduction which entitled the DGAV to ask for annual information about the manufacture and sale of antimicrobial agents for consumption monitoring purposes, was created. Such a legal framework was not in place when the DGAV officially asked that all the authorised wholesalers to retrospectively submit their sales data from 2006 and 2007 by completing a table with information for each year; each antibiotic; their presentations or strengths; the number of packages sold; the quantity of active substance sold; and the location at the country. This information could reveal links between sales data, AMR (human and animal) and the environment [153,193].

The collected information was compiled in a datasheet, and several constraints were detected when the data were received and analysed. These constraints included the following: i) several wholesalers did not reply and others provided information in an anonymous fashion; therefore, the adherent entities and identification of the wholesalers who sold antibiotics could not be specifically accounted for; ii) several datasheets were not fully completed, with arbitrary blanks, due to the inexistence of obligatory fields; iii) the declared sales did not filter intermediate sales among wholesalers to avoid repetitions or to be excluded; iv) several wholesalers included the veterinary products, including antibiotics that were not classified or covered at the time by veterinary legislation; v)
commercial designations of the veterinary medicines were not coincident because part of the name was missing or mistaken or presentations, strengths and formulations, were omitted, partially omitted, or incorrect; vi) the list of active substances and their salts were incomplete or incorrect for the same veterinary medicine; for the same volume of sales of the same veterinary medicine, the calculation of the sold active substance(s) differed a few times, denoting gross lack of accuracy; vii) the UI conversions of several active substance salts into kilograms were mostly absent or random without references; viii) antibiotic classes were linked to the veterinary medicines and not to the active substance(s) and were sometimes incorrect; ix) and the antibiotic class “Others” was not previously defined and therefore, used as arbitrary.

Moreover, several editorial errors in the by hand transcription of the data (approximately 16 750 lines) negatively impacted the data quality. The wholesalers’ receptivity to this initiative was very positive, but the procedure was not simple for the wholesalers and was unprepared and unsupported by appropriated telematics programmes to dispatch the appropriated data or to the DGAV. A robust national database did not exist for authorised veterinary medicines to compare and adequately treat the data. All data were compiled, but the data were not treated until 2011 [198]. For the next step, an IT programm was chosen to treat such a large amount of data, using the VBA (Visual Basic for Applications) software as a Microsoft Office 2007 tool in conjunction with Microsoft Excel after two basic steps of classification and technical grouping of data. Active substances were classified by the ATC (Anatomic Therapeutic Chemical classification) vet System at two distinct groupings: a more specific group until the 5th level (subgroups of chemical substances) and to a more general group at higher levels (third and fourth levels/therapeutic subgroups/pharmacological/chemical), as showed in table 4.
Geographical separation was made on the NUTs [199], in particular NUTS II, to obtain five subdivisions: North, Centre, Lisbon and Tagus Valley (LVT), Alentejo and Algarve. These subdivisions included the respective districts where the veterinary antibiotic medicines were sold. Then, using VBA and adequate numeric techniques that were adapted to modern instrumental techniques of chemical analysis, results and values of quantities of active substances sold for animal use per antibiotic class from 2006 to 2009 were obtained for the first time in Portugal for the veterinary sector.

Table 4 – Veterinary medicine classes and respective ATC vet code as adopted for model 2006/2007.

<table>
<thead>
<tr>
<th>Class</th>
<th>ATC vet Code</th>
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<tr>
<td>Aminoglicosids</td>
<td>QJ01G</td>
</tr>
<tr>
<td>Amphenicols</td>
<td>QJ01BA</td>
</tr>
<tr>
<td>Penicillins/Beta-lactamics</td>
<td>QJ01C</td>
</tr>
<tr>
<td>Cephalosporines</td>
<td>QJ01D</td>
</tr>
<tr>
<td>Lincosamides</td>
<td>QJ01FF</td>
</tr>
<tr>
<td>Macrolides</td>
<td>QJ01FA</td>
</tr>
<tr>
<td>Other antibacterial</td>
<td>QJ01XX</td>
</tr>
<tr>
<td>Other medicines</td>
<td>-</td>
</tr>
<tr>
<td>Pleuromutilines</td>
<td>QJ01XQ</td>
</tr>
<tr>
<td>Polymixines</td>
<td>QJ01XB</td>
</tr>
<tr>
<td>Quinolones</td>
<td>QJ01M</td>
</tr>
<tr>
<td>Sulfonamides</td>
<td>QJ01EQ</td>
</tr>
<tr>
<td>Tetracyclines</td>
<td>QJ01AA</td>
</tr>
<tr>
<td>Trimethoprim and derivatives</td>
<td>QJ01EA</td>
</tr>
</tbody>
</table>

However, the evaluation of the antibiotic consumption in veterinary medicine, based on the sales data, may not coincide with the actual use because sales do not guarantee full usage of package contents; the drug can be used differently from its indications or prescription; human antibiotics could be used in animals; or multispecies presentation,
including food producing animals and/or companion animals, may occur. Due to the reasons listed above, this particular data analysis is challenging.

2.2. ESVAC Model

Between 2008 and 2009, the EEA felt that surveillance programmes of antimicrobial consumption were in need; nine countries had already implemented and tested systems in their countries while few others had even initiated prototypes, like Portugal for years 2006 and 2007. Several other countries lacked national programmes for the veterinary sector or knowledge of antibiotic use in food producing animals [100] until the first ESVAC request on sales data.

The intention of the retrospective collection of sales data was to efficiently obtain a sales pattern along with all of the information that can be gained from the data and used within a national alliance of the veterinary sector in conjunction with the human sector in 2011 [200]. Since then, the sales data in Portugal have been collected in a stratified model according to regional districts and in line with the legal system of veterinary medicine commercialization and other specific regulations.

The data collection form was an Excel datasheet that followed the ESVAC model. This sheet was published on the DGAV website and was available for download by the wholesalers that were previously contacted early in 2012. The wholesalers were kindly asked to complete the form with data from 2008 and 2009 and return it by e-mail. The enforced legislation since 2009 [46] was not retrospective and legal obligation was not posed, but of the 73 active authorised wholesalers in 2012, 55 accepted this request and reported the solicited data. Missing reports were linked to business unrelated to antibiotic veterinary medicines and business occurring outside of 2008 and 2009 or without capacity of data recall for that period. This excel datasheet was user-friendly, and multiple choice
tabs allowed data, such as the final destination of the sold VMP (veterinary medicine products), the number of sold packages, which was approximately 500, and their presentations, to be entered into the sheet.

Sales were considered to be exclusively dispensed from wholesalers to livestock (including aquaculture), veterinarians, veterinary clinics and hospitals, pharmacies (human and veterinary), husbandry cooperatives, farmers organizations and feed mills, who sent the completed data collection forms to the DGAV, excluding sales among wholesalers. The reported variables were those elected by the ESVAC [180] as the data collection form was organized for product information and its ingredients.

Regarding the product, the first grouping included:

The country ISO code identifying the place of collected sales data; the year of the reporting data; the marketing authorisation number of each antibiotic veterinary medicine presentation along the name; the VMP package code value (ID); the VMP name and the codified pharmaceutical form; the pack size unit, as the content unit of measurement allows for the calculation of the amount of active ingredient in each package/product and the ATC veterinary - 5th level. The record of animal species was optional in the ESVAC model.

Regarding each ingredient, (up to four, which is not permitted in Portugal), the second grouping includes: the active ingredient name (ATC vet name), particularly in case of multi-ingredient VMP; the salt of the active ingredient; the prodrug name (ATC vet name); the strength as the quantity of each active ingredient in each unit as declares in SPC/label; the IU conversion factor and the established prodrug factor; and the ingredient content. Many active ingredients are sold individually. Therefore, it is necessary to provide robust national data, specifying the data source/provider.
<table>
<thead>
<tr>
<th>Table 5 - Grouping of 2008/2009 data into antibiotic classes</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Aminoglicosides</strong></td>
</tr>
<tr>
<td><strong>Amphenicols</strong></td>
</tr>
<tr>
<td><strong>Cephalosporines</strong></td>
</tr>
<tr>
<td><strong>Lincosamides</strong></td>
</tr>
<tr>
<td><strong>Macrolides</strong></td>
</tr>
<tr>
<td><strong>Others</strong></td>
</tr>
<tr>
<td><strong>Trimethoprim</strong></td>
</tr>
</tbody>
</table>
After the data were received, they were compiled and inserted into a data collection form that was adapted from ESVAC model and completed with target animal species and regional districts. Antibiotics sold in 2008 and 2009 were grouped into the classes shown in table 5.

3. Results

In 2006 and 2007, over 5 000 Kg per active substance of three main classes of antibiotics, tetracyclines, penicillins/beta-lactams and macrolides, were sold, as shown in table 6.

Table 6 – Kilograms of antibiotic veterinary medicines sold in 2006 and 2007

<table>
<thead>
<tr>
<th>Sales in Kg</th>
<th>2006</th>
<th>2007</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 000 – 6 000</td>
<td>Tetracyclines</td>
<td>Penicillins/Beta-lactamuc</td>
</tr>
<tr>
<td></td>
<td>Penicillins/Beta-lactamuc</td>
<td>Tetracyclines</td>
</tr>
<tr>
<td></td>
<td>Macrolides</td>
<td>Macrolides</td>
</tr>
<tr>
<td>2 000 – 4 500</td>
<td>Sulfonamides</td>
<td>Aminoglicosides</td>
</tr>
<tr>
<td></td>
<td>Aminoglicosides</td>
<td>Sulfonamides</td>
</tr>
<tr>
<td>500 – 2 000</td>
<td>Quinolones</td>
<td>Quinolones</td>
</tr>
<tr>
<td></td>
<td>Polymixines</td>
<td>Polymixines</td>
</tr>
<tr>
<td>&lt; 500</td>
<td>Pleuromutilines</td>
<td>Pleuromutilines</td>
</tr>
<tr>
<td></td>
<td>Cephalosporines</td>
<td>Cephalosporines</td>
</tr>
<tr>
<td></td>
<td>Amphenicols</td>
<td>Amphenicols</td>
</tr>
<tr>
<td></td>
<td>Lincosamides</td>
<td>Lincosamides</td>
</tr>
</tbody>
</table>

The Centre, North and Alentejo regions had higher acquisitions of antibiotic veterinary medicines in 2006 and 2007 [198].
In relation to the 2008 and 2009 data, only AB (antibiotic) classes and respective quantities were evaluated along with the regions in which they were sold. Animal species and pharmacological forms were not analysed.

In 2008, 127.638 tons of antibiotics were sold according to table 7.

Table 7 - Quantities of antibiotic classes sold in 2008

<table>
<thead>
<tr>
<th>Antibiotic classe</th>
<th>Sale quantity (tons)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aminoglycosides</td>
<td>3.004</td>
</tr>
<tr>
<td>Amphenicols</td>
<td>0.682</td>
</tr>
<tr>
<td>Cephalosporines</td>
<td>1.883</td>
</tr>
<tr>
<td>Lincosamides</td>
<td>15.200</td>
</tr>
<tr>
<td>Macrolides</td>
<td>14.306</td>
</tr>
<tr>
<td>Others</td>
<td>0.014</td>
</tr>
<tr>
<td>Penicillines</td>
<td>16.422</td>
</tr>
<tr>
<td>Pleuromutilines</td>
<td>26.499</td>
</tr>
<tr>
<td>Polymixines</td>
<td>3.079</td>
</tr>
<tr>
<td>Quinolones</td>
<td>6.424</td>
</tr>
<tr>
<td>Sulfonamides</td>
<td>5.205</td>
</tr>
<tr>
<td>Tetracyclines</td>
<td>31.763</td>
</tr>
<tr>
<td>Trimethoprim</td>
<td>3.157</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>127.638</strong></td>
</tr>
</tbody>
</table>

From the total sales in 2008, tetracyclines are the most sold class of AB, followed by pleuromutilines and penicillins. Lincosamides, mostly represented by lincomycin, was the fourth most sold antibiotic for veterinary use in 2008, followed by, in descending order, the macrolides, quinolones and sulfamides. Aminoglycosides and cephalosporines exceeded polymyxines, amphenicols and others (rifaximine), respectively.
In 2009, 154.541 tons of antibiotics were sold, according to table 8.

From the total sales in 2009, tetracyclines were the most sold AB, followed by penicillins and lincosamides. Lincosamides, mostly represented by lincomycin, was the third most sold class of antibiotics for veterinary use in 2009, followed by, in descending order, the macrolides, quinolones and pleuromutilines. Sulfamides were exceeded by polymyxines. Aminoglycosides and cephalosporines exceeded trimethoprim, amphenicols and others, respectively.

<table>
<thead>
<tr>
<th>Antibiotic classe</th>
<th>Sale quantity (tons)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aminoglycosides</td>
<td>3.554</td>
</tr>
<tr>
<td>Amphenicols</td>
<td>0.861</td>
</tr>
<tr>
<td>Cephalosporines</td>
<td>3.261</td>
</tr>
<tr>
<td>Lincosamides</td>
<td>19.672</td>
</tr>
<tr>
<td>Macrolides</td>
<td>12.853</td>
</tr>
<tr>
<td>Others</td>
<td>0.011</td>
</tr>
<tr>
<td>Penicillines</td>
<td>23.217</td>
</tr>
<tr>
<td>Pleuromutilines</td>
<td>8.836</td>
</tr>
<tr>
<td>Polymixines</td>
<td>6.367</td>
</tr>
<tr>
<td>Quinolones</td>
<td>10.409</td>
</tr>
<tr>
<td>Sulfonamides</td>
<td>4.462</td>
</tr>
<tr>
<td>Tetracyclines</td>
<td>58.594</td>
</tr>
<tr>
<td>Trimethoprim</td>
<td>2.444</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>154.541</strong></td>
</tr>
</tbody>
</table>
In the LVT region, all classes of AB (except others) were sold in the greatest quantities, followed by Centre, North, Alentejo (2008 – figure 11) or by Centre, Alentejo, North (2009 – figure 12) and Algarve, where the smallest amount of antibiotics were acquired either in 2008 and 2009.

**Figure 11** - Antibiotic classes sold by regions in 2008

**Figure 12** - Antibiotic classes sold by regions in 2009
The slight increase in antibiotics sales during 2009 (figure 13) was also verified for the major three classes of AB: Tetracyclines, Penicillins and Lincosamides.

![Figure 13 – Veterinary antibiotic sales comparison between years 2008 and 2009]

Finally, the most sold antibiotics in Portugal in 2008 and 2009 and the animal species, for which the AB is indicated, according the respective SPC, are represented in table 9.

**Table 9 - Quantities and target species of the antibiotic mostly sold in 2008 and 2009**

<table>
<thead>
<tr>
<th>Antibiotic classe</th>
<th>Most sold</th>
<th>2008 (tons)</th>
<th>2009 (tons)</th>
<th>Target species</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tetracyclines</td>
<td>Oxytetracycline</td>
<td>17.007</td>
<td>31.501</td>
<td>Multispecies</td>
</tr>
<tr>
<td>Pleuromutilines</td>
<td>Tiamulin</td>
<td>20.711</td>
<td>8.210</td>
<td>Porcine, Poultry</td>
</tr>
<tr>
<td>Penicillins</td>
<td>Amoxicillin</td>
<td>12.912</td>
<td>18.013</td>
<td>Multispecies</td>
</tr>
<tr>
<td>Lincosamides</td>
<td>Lincomycin</td>
<td>15.198</td>
<td>19.670</td>
<td>Porcine</td>
</tr>
<tr>
<td>Macrolides</td>
<td>Tylosin</td>
<td>11.795</td>
<td>10.604</td>
<td>Porcine, Poultry</td>
</tr>
<tr>
<td>Quinolones</td>
<td>Enrofloxacin</td>
<td>5.613</td>
<td>7.481</td>
<td>Dogs, Cats, Bovine, Poultry</td>
</tr>
<tr>
<td>Polymixines</td>
<td>Colistin</td>
<td>6.367</td>
<td>3.079</td>
<td>Porcine</td>
</tr>
<tr>
<td>Cephalosporins</td>
<td>Cefalexin</td>
<td>1.631</td>
<td></td>
<td>Dogs, Cats</td>
</tr>
<tr>
<td>Cephalosporines</td>
<td>Ceftiourfur</td>
<td>2.090</td>
<td></td>
<td>Porcine, Bovine</td>
</tr>
</tbody>
</table>
4. Discussion

The ESVAC model identifies which pharmaceutical forms are more often used and allows the dimensions and patterns of AB use to be examined, namely at regional level, where livestock are located. For example, premixes and oral powders are used in feed for prophylaxis, metaphylaxis and therapy in groups of animals as mass medication. However, the quantities of medicated feed acquired from another MS by free Community trade [22] is not reflected in premixes sales and medicated feed but may be an important, if not predominant, in the administration of antibiotics to food producing animals. Although the destination MS may require that each consignment of a medicated feed be accompanied by a certificate issued, and this document could be used to sum up local acquisitions for consumption.

Livestock locations, animal species and the type of production and knowledge about AB consumption, along with information about human health facilities and environment, will enable examination of the link to the impact on AMR. This is an established “One Health” aim in Portugal, where veterinarians may dispense antibiotics as part of their service but are not allowed to trade veterinary medicines [46].

Patterns and trends on AB veterinary sales are becoming identifiable, and patterns and trends of consumption are now the main issue to enable a more accurate epidemiological analysis of AMR in both the human and veterinary sectors after appropriate measurement units have been established. A valuable measurement unit for veterinary AB consumption has to be sustained by adequate information regarding the target population. Different AB strengths may vary considerably, depending on their own potency, pharmacokinetic characteristics, formulations, minimum inhibitory concentrations and the infection itself. To adjust such strengths and differences in several veterinary antibiotics and respective formulations and to facilitate any comparisons between populations, data on animal
species use must be calculated as numbers and expressed as administered daily doses, as animal DDD.

Until now, to link the quantities of veterinary AB sold with animal demographics per country, the population correction unit has been calculated and used as the term for the estimated animal weight. It is a technical unit of measurement that estimates the sales corrected by the animal population in countries and across countries and it is calculated from Eurostat data [180], which currently has several inexistent statistical figures relating years 2006/2009. However, proportionally, the sales figures for this period, and annually, did not diverge significantly from others collected in several EU MS with similar animal production profiles [124].

Nevertheless, the data should be robust. Wholesalers are not individually inspected to detect any possible errors or inaccuracies in their data collection and data transmission. Additionally, the products that they sell may not accurately represent the drugs that are consumed, due to either leftover drugs or parallel markets. In Europe, to complicate this issue, the commercialization of veterinary medicine is not standardised, and the market channels are established as determined by the MS to best serve the health of its animals.

Currently, all veterinary AB should be prescribed in light of the EU legislation. Prescriptions could be one of the most reliable sources of animal consumption data, at least for food producing animals, if electronic prescriptions were compulsory. On a predefined collection form, veterinarians of companion animals should annually report their purchased antibiotics, and pharmacies should report their sales under veterinary prescriptions. This procedure would also serve to control veterinary medicine legislation regarding legal trade and food safety.

Without any complementary information from prescribers, the shift of total sales over the years and deviation of several AB classes at the same period of time must be carefully
interpreted. This is because it is generally agreed that it takes at least three to four years to establish a valid baseline for the data on sales of VMP due to treatment of companion animals with human AB and veterinary injectable AB and treatment of food producing animals via the “cascade system”. Where veterinary medicines for humans or animals may be legally used in animal species other than those authorised (in Portugal, since 2011 [201]) at the farm level only, veterinarians are obliged to declare if and how they use “cascade system”, especially because sales figures should be matched and compared with many other factors.

Increasing and/or decreasing sales values over the years may have several meanings, considering that the pet population is far more stable and the number of food producing animals may increase or decrease with or without proportional variation on livestock numbers within a heavier economical context. Biosecurity, geographical location, economic viability, the type of production, consumer demands, and veterinary epidemiology will be the trigger and balance of veterinary medicine sales. In animal production, no antibiotic is used without a specific return, and currently, it is important to include the calculated risk of AMR to an AB in a prudent manner.

Both collection data systems on AB sales in study are key steps of the model, but robust data may lead to enhanced quality of both the data itself and the results obtained from the data when used by regulators or scientists within the framework of AMR epidemiology. However, veterinarians prescribing, using the medications and avoiding misuse and abuse; pharmacists selling veterinary medicines; and the responsible cooperation of all animal owners and animal producers and general public are important for achieving the “One Health” tribute, as an interdisciplinary collaboration converging human disease, animal disease and a common approach to biosecurity [12,189,202].
5. Conclusions

The ESVAC system of data collection used for 2008/2009 was much easier and more efficient, readable and useful for science, policy and decision makers. Information about which AB substances are sold at the highest rates can suggest which species consume more quantities of AB.

MS should only compare ESVAC data in a very holistic manner and taking into account all concurrent national factors and particularities reflected in the country-to-country differences in resistance as a function of several parameters, namely those relating to differing management systems, disease incidence and antimicrobial usage and avoiding unnecessary misinterpretations [203,204].

In conclusion, the present study shows that:

Reliable data on the sales of veterinary antibiotics were presented, or the first time, in Portugal; Antibiotic animal consumption data cannot yet be evaluated based on sales only, as any other rigorous interpretation of this deviates; Data on veterinarian prescriptions, preferably collected by electronic format, should be encouraged not only for accuracy reasons but also because prescription data better represent real use than wholesaler’s declarations; Robust, accurate and reliable sales data within an established baseline used in an AMR epidemiology context should allow breakthroughs in collecting AB consumption data; Patterns, prevalence and trends compared with quantities of AB consumed by species, with an appropriate unit of measurement for veterinary AB medicines, will certainly enable a more accurate comparison between animal and human use of AB, which is currently an important issue in Europe [153,205,206].

Thus, this paper makes an important contribution to improving the knowledge about antibiotic usage in Portugal, particularly for studies of antibiotic resistance.
CHAPTER II

ANTIMICROBIAL CONSUMPTION BY ANIMAL SPECIES

Antimicrobial Consumption in Swine in Portugal: Estimation of the Population Factor to Monitor Antimicrobial Resistance

Abstract

The establishment of technical units of measurement for the determination of species-specific antimicrobial consumption in animals, such as the DDDvet and the DCDvet, previously proposed by the ESVAC (European Surveillance of Veterinary Antimicrobial Consumption), may facilitate drug consumption studies to assist the reduction of selective pressure on resistant bacteria emergence [114, 175], decreasing antimicrobial resistance (AMR) development and spread. Resistance and antimicrobial consumption monitoring are key factors to improve the surveillance of antimicrobial resistance and implement the most adequate management measures to control it. Besides the AMR-related public health concerns, common to humans and animals, food safety criteria regarding the use of antimicrobials in animals, for trade animals and food of animal origin purposes, usually have a major role in the country’s economies [207], requiring international agreement on consumption measurement and report, of individual and overall antimicrobial consumption across countries, with impact on global markets. The ESVAC antimicrobial data consumption collection model by species was tested in approximately one-third of the Portuguese swine population in 2013. However, the determination of “annual pig production”, which was estimated from a “population sample” after extrapolation to the “national population” and combined with animal treatments observations, revealed some specificities that should be revisited and further explored [18, 208]. The present study intends to contribute to the refinement of the proposed ESVAC model in relation to the
population factor in swine and to serve as a basis for a national surveillance system integrated with AMR data, as determined by resistance levels in 21 *Escherichia coli* strains isolated from pigs, in the same year.

1. Introduction

Antimicrobial resistance (AMR) is a regulatory concern in the veterinary field because despite some molecules have been introduced for veterinary medicine only like, Apramycin, Florfenicol, Tylosin, Tilmicosin and Tiamulin [154], almost all veterinary antimicrobial medicines are structurally related to antimicrobials for human use and may, therefore, select for bacterial resistance [147,175]. This scenario has become rather serious, as the availability of veterinary antimicrobial medicines tends to maintain very low [1,9,114,154]. Recently, the European Commission (EC) asked the EMA (European Medicines Agency) to develop a harmonized approach to collect and report veterinary data based on national antimicrobial sales figures combined with estimates of antimicrobial usage in animal species to ensure comparability with human antimicrobial use data [195]. A sales data collection model for veterinary antimicrobial medicines was initially implemented in 2011 [124] by the ESVAC (European Surveillance of Veterinary Antimicrobial Consumption) project which activity started in 2009 [18] and thereafter improved and proceeded in 2014 by a pilot phase on to collect antimicrobials consumption data in pigs; however, this pilot phase was suspended after one year’s duration [18]. Data from antimicrobial sales may not reflect species-specific consumption patterns, as most veterinary antimicrobial medicines are multispecies. A consumption data collection model should enable the calculation of the veterinary antimicrobials consumed by an animal species and aggregated by their different life cycles over the distinct production stages, in a certain region or country and for a determined period of
time. It should also ensure comparability between the use of antimicrobials in animals and humans by establishing DDDvet and DCDvet units harmonized, to whatever extent possible, with the assigned DDD units for human medicines consumption measurement. Some countries already monitor veterinary antimicrobial consumption using their own data collection models, data sources and technical units of measurement [18,209,210,211]. In Portugal, veterinary antimicrobial consumption has never been monitored; however, an Annual National Control Plan of Medicines Use has been implemented in farms, through a documental traceability system and including records on antimicrobials usage. Veterinarians are the only professionals licensed to prescribe medication (medicines and medicated feed) for animals in Portugal and, in the case of food-producing animals, official prescriptions are issued in triplicate (for the retailer, farmer and prescriber). Complementary, veterinary requisition papers, serving as list of the medicines to be acquired and regularly available at the farm, are also issued in duplicate by the veterinarian, who keeps a copy while the original is kept by the wholesaler/retailer. At the farm, all used medicines are registered [46,47] in an appropriate book or adequate information technology system and crosschecked with prescriptions, requisitions, receipts and stocks in farms and wholesalers. [46] However, this official control system is not a practical approach to collect dispersed data on veterinary antimicrobial medicines consumption, considering that the data sources are farms and not health facilities, despite incubated, diseased and healthy animals are present all the time [117]. Furthermore, profound changes in farm structures and on professional mentalities are needed to facilitate data collection in feasible and accurate format [18]. By complying with the higher animal welfare standards, pigs have been raised in increasingly better health conditions [212,213], and farmers associations (FPAS) claim to have reduced overall antibiotic consumption in Portugal. Recent reforms in this sector
concentrated the largest percentage of national swine population into a few groups of holders, with substantial decrease of the smallest farms. This study reports an analysis of the veterinary medicine’s data consumption in five pig producer groups with a total of 101 farms, exclusively supplied by a cooperative retail system. As in the ESVAC reports, medicated premixes national sales [125] are not indicative of the medicated feed national consumptions, it was decided to restrict this study to oral and injectable formulations. Additionally, it was assumed that all acquired medicines were fully administered.

There are several factors [128] that contribute to the acceleration of AMR development, resulting in increased infectious disease rates in both humans and animals, such as environmental pollution and degradation, larger population concentrations, animal production intensification [113], human demographic changes, old and new immune deficient diseases (infectious, allergic and oncologic) [114], world globalization, large scale use of antimicrobials for treatment and prophylaxis, self-medication and prescription pressure. The control of enteric bacterial diseases commonly associated with high morbidity/mortality rates and heavy economic losses is pig production industry and that may be potentially pathogenic to both humans and animals, is a critical goal [207,212,214]. Monitoring of antimicrobial resistance plays therefore an important role in the evaluation and management of the implemented antibiotic consumption surveillance measures [1]. This paper discusses the use of a population-based model for the evaluation of reported veterinary antimicrobial medicines consumption data by animal species in a representative sample of swine and highlights the importance of accurate data when comparing antimicrobial consumption with resistance data in animals [18] and publishing country-specific veterinary antrimicrobials consumption profiles.
2. Material and methods

2.1 VAB Data

Five swine producer groups representing almost one-third of the national pig population participated in this study in 2013. To decreases costs with veterinary medicines, this consortium owns an exclusive supplier, which provided the antimicrobials sales data for this study, including the monthly dispensed packages, aggregated by three different stages of pig production (piglets, sows and fattening pigs). Using the 2013 ESVAC antimicrobials sales data collection template [18,197,215,216], only the names of those antimicrobials were maintained in their original fields, and the respective number of sold packages were inserted in the corresponding columns to determine the quantity (in tons) of each active substance sold/consumed throughout the pig life cycles.

The vast majority of these farms were located in LVTR (Lisbon and Tagus Valle Region). Data on human antimicrobial consumption in this region during the same year was provided by the INFARMED – National Authority of Medicines and Health Products, I.P., in DDD and in number of prescribed packages; these figures were converted into tons according to the formulation and strength of the prescribed medicines for human use.

In LVTR, the total amount of antimicrobial consumption was estimated to be 23.699 tons in humans (INFARMED) and 14.274 tons in swine (data from the DGAV – Portuguese Animal Health Authority) in 2013. Humans did not consume any amphenicols, pleuromutilins or polymyxins, while pigs did not consume any anti-tuberculosis agents, monobactams and antimicrobials classified in the human sector as “others” during that time period.

In decreasing order, the top three antibiotics most frequently consumed by swine were lincomycin (7.661 tons), amoxicillin (2.499 tons) and doxycycline (1.308 tons), while the
top three antibiotics used by humans were amoxicillin (15.659 tons), ciprofloxacin (1.934 tons) and clarithromycin (1.018 tons).

Comparisons between human and pig antibiotic consumption per pharmacological class, in LVTR during 2013 are shown in table 10.

<table>
<thead>
<tr>
<th>Pharmacological classes</th>
<th>Human Consumption (ton)</th>
<th>Swine Consumption (ton)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AMINOGLYCOSIDES</td>
<td>0.001</td>
<td>0.473</td>
</tr>
<tr>
<td>CEPHALOSPORINS</td>
<td>1.736</td>
<td>0.080</td>
</tr>
<tr>
<td>LINCOSAMIDES</td>
<td>0.001</td>
<td>7.661</td>
</tr>
<tr>
<td>MACROLIDES</td>
<td>1.833</td>
<td>0.391</td>
</tr>
<tr>
<td>PENICILLINS</td>
<td>17.274</td>
<td>2.621</td>
</tr>
<tr>
<td>QUINOLONES</td>
<td>2.696</td>
<td>0.381</td>
</tr>
<tr>
<td>TETRACYCLINES</td>
<td>0.146</td>
<td>1.537</td>
</tr>
</tbody>
</table>

2.2 Swine Population Data

All farms was identifiable at the regional level by an official unique digit code, as required by the additional guarantees regulation provided for the swine intra-community trade [217]. The veterinarians in charge identified each farm’s location and the aggregated swine populations per life cycle in 2013; these were, in total, as follows: 192,193 piglets, 131,979 fattening pigs and 42,998 sows. Information regarding the number and category of pigs in farms are mandatory and submitted as official notifications to the DGAV three times a year. After validation of the farm identity, sub-population figures were crosschecked with the corresponding official data from the DGAV databases and
analysed to determine production variables such as repopulation and sow replacement rates and offspring averages. Regionally, the 101 farms included in this sample were in 38 different locations defined by the Portuguese NUTs II- Territory Units Nomenclature [218]; of the farms, 12 were in Alentejo, 16 were in Centro and 73 were in the LVTR. Of the farms, 62 were farrow-to-finish farms, 29 were farrow-to-wean, 7 were fatteners only, 2 were a combination of fatteners and farrow-to-wean, and 1 was sows only.

2.3 Resistance Data
As representatives of the family Enterobacteriaceae, commensal Escherichia coli have been frequently selected as indicators of antimicrobial resistance in Gram-negative bacterium usually present in animal feces. Additionally, E.coli often affect primarily the younger pigs [207]. Present in the intestines of farm animals, [214] E. coli have plasmid resistance genes that can often be transferred between enteric bacteria and spread horizontally to zoonotic and other bacteria present in the food chain. E. coli is also relevant to human health [114,121,219,220]. Although the zoonotic bacteria Salmonella remains the most frequently reported cause of foodborne outbreaks in the EU [117,155,157,221], commensal E. coli and its pathogenic variants have been identified as responsible for serious infections that require different therapeutic approaches in humans and animals, not dispensing antibiotic therapy in many cases. The monitoring of antimicrobial resistance in E. coli isolated from either randomly selected animals or their associated carcasses and meat has been identified as a mechanism by which to data representative of the general population is obtained; additionally, these data provide valuable information regarding the occurrence of resistance in evaluated populations and may help to monitor the emergence and changes in the proportion of bacteria possessing ESBLs (extended spectrum beta-lactamases) [121,222]. In 2013, only 10 Member States
(MS) and Switzerland provided quantitative antimicrobial resistance data for indicator strains of *E. coli* in pigs to the EC; however, these data were not categorized by production type. The majority of MSs collected isolates as part of their national antimicrobial resistance monitoring programmes, and these data were mostly derived from random sampling of healthy pig carcasses at slaughterhouses. Overall, detailed information on sampling stages and contexts and sample types were missing, and “microbiological” resistance was assessed using epidemiological cut-off (ECOFF) values.

Substantial differences were noted in the occurrence of resistance between different MSs; however, among the MSs, the highest overall ‘microbiological’ resistance levels were observed against tetracyclines (52.8 %), streptomycin (47.8 %), sulfonamides (42.1 %) and ampicillin (30.3 %).

**Table 11** - Information related to antimicrobial resistance of 21 *E. coli* isolates obtained from swine, suspected from enteric pathology in Portugal, in 2013

<table>
<thead>
<tr>
<th>Nº OF ISOLATES</th>
<th>AMO</th>
<th>APR</th>
<th>AMP</th>
<th>CEF</th>
<th>COL</th>
<th>DOX</th>
<th>ENR</th>
<th>FLU</th>
<th>GEN</th>
<th>LIN</th>
<th>MARB</th>
<th>NEO</th>
<th>OXI</th>
<th>SULFAZOL +TRI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resistent</td>
<td>15</td>
<td>14</td>
<td>7</td>
<td>0</td>
<td>8</td>
<td>19</td>
<td>11</td>
<td>10</td>
<td>6</td>
<td>21</td>
<td>3</td>
<td>2</td>
<td>5</td>
<td>18</td>
</tr>
<tr>
<td>Intermediate</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Susceptible</td>
<td>3</td>
<td>5</td>
<td>0</td>
<td>5</td>
<td>11</td>
<td>2</td>
<td>10</td>
<td>9</td>
<td>14</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>No info.</td>
<td>3</td>
<td>0</td>
<td>14</td>
<td>15</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>16</td>
<td>16</td>
<td>16</td>
<td>2</td>
</tr>
<tr>
<td>Total</td>
<td>21</td>
<td>21</td>
<td>21</td>
<td>21</td>
<td>21</td>
<td>21</td>
<td>21</td>
<td>21</td>
<td>21</td>
<td>21</td>
<td>21</td>
<td>21</td>
<td>21</td>
<td>21</td>
</tr>
</tbody>
</table>
Resistance to ciprofloxacin and nalidixic acid was low (6.1 % and 3.8 %, respectively), and only 1.3 % of isolates were resistant to cefotaxime. Resistance in *E. coli* has not been monitored by the Portuguese Surveillance Program. Nevertheless, antimicrobial resistance data for 21 *E. coli* strains isolated from swine suspected to have enteric illness in the Portuguese mainland territory in 2013 are provided in table 11.

3. Results

3.1 The Population Sample

To provide valid estimates of national consumption aggregated by pig production stage, a cross-sectional study with a single data collection event was conducted using a questionnaire that determined the number of animals born, raised and/or maintained on the 101 study farms based on retrospective data collected in 2013. Those figures were then crosschecked with the official information [217] submitted to the DGAV regarding these pig populations. After identification of the farms to which the veterinary antimicrobials had been sold, the pig national population was calculated for each stage of the pig production using a simple demographic model based on Portuguese national figures and considering that fattening pigs weight 70 Kg and sows weight 220 Kg. In Portugal, piglets are commonly bred either to be weaned and fattened (in one or more herds) or to be consumed as piglet meat at a very early stage, depending on the region in which it is to be consumed. A piglet’s permanence in the herd is therefore variable; for this reason, a 10 Kg standard bodyweight (BW) was defined for all piglets, regardless of the productive endpoint.
3.1.1 Fattening pigs

Based on the official population submitted to the DGAV (131,979) multiplied by the time period of the evaluation (365 days) and divided by a gestational period of 113 days, the average production of fattening pigs was estimated to be 426,304 head per year. The total sample population was considered to be the average of the monthly number of fattening pigs of which the DGAV was officially notified during a given year (1,066,025). Based on the total sample population multiplied by the time period of the evaluation (365 days) and divided by a gestational period of 113 days, the national average population was 3,443,355 head. The ratio of the sample to the overall population was 12 % (426,304/3,443,355).

3.1.2 Piglets

Based on the official population submitted to the DGAV multiplied by the time period of the evaluation (365 days) and divided by the average life period of 60 days, the estimated average production of piglets was 1,169,174 head per year. The total sample population was considered to be the average of the monthly number of piglets of which the DGAV was officially notified during a given year (652,165). Based on the total sample population multiplied by the time period of the evaluation (365 days) and divided by the average life period of 60 days, the national average population was 3,967,377 head. The ratio of the sample to the overall population was 29 % (1,169,174/3,967,377).

The annual average production of piglets could have, however, been estimated assuming that the number of piglets produced per sow per year was 24, and in this case, the results would have been rather different, with an annual average production of 1,031,952 piglets based on the population of sows of which the DGAV was officially notified (42,998)
multiplied by 24. The total sample population, average national population and antimicrobial consumptions would also have differed based on this estimate.

3.1.3 Sows

The estimated average production of sows was 42,998 head per year, which was the same as the officially notified number because this subpopulation is usually the most stable, with a lifespan of 3 years and an average annual repopulation rate of 40 %. The total sample population was the average of the monthly number of sows of which the DGAV was officially notified during a given year (185,656 animals). The ratio of the sample to the overall population was 23 % (42,998/185,656).

3.2 - VAB consumption Data

In accordance with the collected veterinary antimicrobials sales data from 2013, a total of 1.665 tons of antibiotics were consumed by piglets, 2.159 tons of antibiotics were consumed by fattening pigs and 0.104 tons of antibiotics were consumed by sows (Table 12). The frequencies of oral and injectable pharmaceutical formulations used during each stage of pig production are shown in Figure 14.

![Figure 14](image_url)

**Figure 14**- Percentage of oral and injectable forms of veterinary antibiotics consumed by the population sample, per pig life cycles in 2013.
**Table 12** - Quantities (in tons) of active substances consumed, by the population sample, per pig life cycles in 2013.

<table>
<thead>
<tr>
<th>VAB Active Substance</th>
<th>Piglets</th>
<th>Sows</th>
<th>Fattening Pigs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amoxicillin</td>
<td>1.190</td>
<td>0.057</td>
<td></td>
</tr>
<tr>
<td>Benzylpenicillin</td>
<td></td>
<td>0.004</td>
<td></td>
</tr>
<tr>
<td>Ceftiofur</td>
<td>0.025</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Colistin</td>
<td>0.190</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dihydrostreptomycin</td>
<td></td>
<td>0.014</td>
<td></td>
</tr>
<tr>
<td>Doxycycline</td>
<td></td>
<td></td>
<td>0.298</td>
</tr>
<tr>
<td>Enrofloxacin</td>
<td>0.127</td>
<td>0.028</td>
<td></td>
</tr>
<tr>
<td>Florfenicol</td>
<td>0.110</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lincomycin</td>
<td></td>
<td>0.003</td>
<td>1.334</td>
</tr>
<tr>
<td>Marbofloxacin</td>
<td></td>
<td>0.030</td>
<td></td>
</tr>
<tr>
<td>Oxytetracycline</td>
<td>0.021</td>
<td>0.021</td>
<td></td>
</tr>
<tr>
<td>Spectinomycin</td>
<td></td>
<td>0.006</td>
<td></td>
</tr>
<tr>
<td>Sulfadimethoxine</td>
<td>0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tiamulin</td>
<td></td>
<td></td>
<td>0.256</td>
</tr>
<tr>
<td>Trimethoprim</td>
<td>0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tylosin</td>
<td></td>
<td></td>
<td>0.213</td>
</tr>
</tbody>
</table>

**3.3 - Extrapolation to National Swine Consumption**

Considering the estimated veterinary antimicrobials consumption data for 2013, the extrapolated amount of antibiotics consumed by the national swine population of Portugal (around three-fold higher) in 2013 was 5.649 tons in piglets, 17.435 tons in fattening pigs and 0.451 tons in sows (Table 13).

The quantities of antimicrobial agents consumed were based on the population factor. Regarding the veterinary antimicrobial medicines in this study, comparisons of total national VAB sales in 2013 (Fifth ESVAC Report) and the estimates for the number of antibiotics sold and consumed (in tons) within the study sample population and the extrapolated national swine population are shown in Figure 15.
Table 13 – Tons of active substances consumed, by extrapolation, during the life cycles of pig production in Portugal in 2013.

<table>
<thead>
<tr>
<th>VAB Active Substance</th>
<th>Piglets</th>
<th>Sows</th>
<th>Fattening Pigs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amoxicillin</td>
<td>4.038</td>
<td>0.246</td>
<td>6</td>
</tr>
<tr>
<td>Benzylpenicillin</td>
<td></td>
<td>0.018</td>
<td></td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>0.084</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Colistin</td>
<td></td>
<td>0.643</td>
<td></td>
</tr>
<tr>
<td>Dihydrostreptomycin</td>
<td></td>
<td>0.059</td>
<td></td>
</tr>
<tr>
<td>Doxycycline</td>
<td></td>
<td>2.403</td>
<td></td>
</tr>
<tr>
<td>Enrofloxacin</td>
<td>0.431</td>
<td>0.226</td>
<td></td>
</tr>
<tr>
<td>Florfenicol</td>
<td>0.374</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lincomycin</td>
<td></td>
<td>0.013</td>
<td>10.772</td>
</tr>
<tr>
<td>Marbofloxacin</td>
<td></td>
<td></td>
<td>0.244</td>
</tr>
<tr>
<td>Oxytetracycline</td>
<td>0.071</td>
<td>0.091</td>
<td></td>
</tr>
<tr>
<td>Spectinomycin</td>
<td></td>
<td>0.025</td>
<td></td>
</tr>
<tr>
<td>Sulfadimethoxine</td>
<td>0.006</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tiamulin</td>
<td></td>
<td></td>
<td>2.066</td>
</tr>
<tr>
<td>Trimethoprim</td>
<td>0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tylosin</td>
<td></td>
<td></td>
<td>1.723</td>
</tr>
</tbody>
</table>

Figure 15 - Proportion of antibiotic active substance total sales and quantities (in tons) consumed by pigs (study sample and extrapolated national pig population) in Portugal in 2013.
4. Discussion

In 1996, the World Health Organization (WHO) declared the need for the use of a classification system, called the ATC/DDD (Anatomical Therapeutic Chemical /Defined Daily Dose), as an international standard for drug utilization studies; subsequently, the ATCvet classification was implemented [163] in 2001. The human DDD provides a rough estimate of the consumption of medicines, allowing for the assessment of trends and performance of comparisons between population groups, and it is nearly always a stable estimate based on a review of the available information [223,224,225]. Similar standards have also been developed in the veterinary sector [18]. Currently, trends in veterinary antimicrobials sales are linked to annual animal demographics in each MS, enabling calculation of the Population Correction Unit (PCU) by species, age class and production type [128]. Quantitative comparisons of active substances consumed per biomass treated (animal and human) may provide regulators with a general view of usage trends in both sectors [142]; however, this analysis is not conclusive, for guidance on prudent use in the different animal species and life-cycles [147,175] Reporting animal consumption of VAB in the form of DDDvet or DCDvet [18] may, therefore, represent a substantial improvement over the reporting of the consumption of active substances in tons. Actually, levels of critically important antibiotic consumption that are quantitatively lower than the levels of consumption of other less critical classes of antibiotics do not necessarily represent a lower hazard associated with AMR in either the human or the veterinary sectors. While the use of related, more potent substances or some critical classes of antibiotics in smaller and lighter animals (like piglets) for treatment may give the false impression of a decrease in total drug consumption, treatment intensity might be unchanged or even increased. The selective pressure that antimicrobials exert on microorganisms is not only proportional to the quantities of antimicrobials consumed.
Many attempts have been made to establish harmonized technical units of measurement to quantify antimicrobial consumption in animals. Some countries have created these metrics in their own surveillance systems [147], establishing, for instance, the ADD (the system of animal defined daily doses, considered as the precursor of the international veterinary technical unit); however, regarding the differences in dose and main indications established for VABs no agreement was ever achieved [163,225] between countries. It has also been recognized that units, including the ADD and others such as the UDD (Used Daily Dose), the PDD (Prescribed Daily Dose) or the TI (Treatment Impact), [225,226,227] do not represent real veterinary antimicrobials consumption, and although these units may allow comparisons at national level, it cannot be used to even evaluate trends among countries [120,147,186,211,228]. Indeed, different means of adjusting usage estimates based on the number of animals available for treatment may create additional risk of inconsistency and disagreement when reporting antimicrobial consumption [186]. When applying the ESVAC principles [229] to calculate animal data consumption, which is the basis for the assignment of DDDvet and DCDvet units, the “population factor” has been identified as a barrier to their calculation requiring further harmonization in its calculation methods to overcome the detected difficulties [186] and avoid major constraints when the authorities are called upon to report and publish results on antimicrobial consumptions in animals. Essentially, only the implementation of an electronic veterinary prescription system [18] with continuous and automatic data collection is believed to be able to correctly address and streamline the procedures for collecting, totaling, and calculating consumption data for a given population. A pilot phase of the pig antimicrobial consumption data collection implemented by the ESVAC has been conducted in a few MSs; however, this test has run on full cycle farms only and included a minimum number of farms instead of a minimum number of animals [18,208].
Some MSs were not eligible to participate in that phase due to a lack of legal support, human and economic resources or representability of its swine farms. Applying this model to the context of the Portuguese swine production, considerable difficulties have been identified in determining the most appropriate sample population to be used for extrapolation at the national level; this has, in particular, be difficult in consideration of the duration of each life cycle because “age-groups” represent, to some extent, the animal traditional production classes in different countries or regions [212]. Moreover, animal weights vary broadly across various breeds and meat consumer preferences. Further improvements should also include the development of criteria for the determination of a representative population sample, as farm dimensions may be very distinct between countries and within regions. Some specific productive factors such as birth, morbidity, mortality and repopulation rates and “all-in/all-out” length and frequencies should also be considered when determining a population sample. In addition, appropriate indicators such as bacterium isolates and resistance data may be associated with significant changes in veterinary antimicrobial medicines consumption in the epidemiological context of animal diseases and outbreaks [208,221].

During the determination of the veterinary antimicrobials classes consumed by pigs during each stage of production in the present study (Tables 3 and 4), critically important antibiotics used in human medicine such as cephalosporins (ceftiofur), polymyxins (colistin) and amphenicols (florfenicol - a compound related with the forbidden chloramphenicol) were observed to be consumed by piglets only, suggesting the need for a major focus on this stage of the pig life cycle during which management is crucial and complex [212]. The number of piglets per litter is often a continuous challenge during pig production often involving, over short intervals considerable movement of many animals.
between and within herds and farms and resulting in emergent risks of stress-associated pathology and recurrent use of critical antibiotic classes [175].

Within the available data regarding resistance in Portuguese swine (table 2) not divided by production stage, it became evident that all of the 21 E. coli isolates were resistant to lincomycin, which was the most frequently consumed antibiotic by fattening pigs and by sows during 2013 (table 4). Nineteen isolates were resistant to doxycycline, which was the third most frequently consumed VAB in fattening pigs, while oxytetracycline was less frequently consumed, and resistance to this agent was observed in 5 isolates only. Eighteen E. coli isolates were resistant to sulfamethoxazole/trimethoprim; however, consumption of that particular sulfonamide was not identified in the population studied. Despite the probability of a resistance pattern potentially similar to that observed in sulfonamides overall, it was concerning that while these two substances were the less frequently consumed in piglets, resistance to these agents was observed in a high number of isolates. These resistance finding may be associated with some type of cross resistance but also may be associated with illegal use (not prescribed; not registered) or due to predominant administration via medicated feed, which was not assessed in this study. Fifteen E. coli isolates were resistant to amoxicillin, which was the second most frequently consumed veterinary antimicrobials in piglets and sows, and although 7 isolates were resistant to ampicillin, this active substance was the third least consumed antimicrobial in sows. The inversion of the levels resistance expected in amoxicillin and doxycycline and fact that in these agents, resistance was not directly associated with their respective consumption levels may be explained, again, by a lack of data regarding medicated feed intake, which is a very common vehicle of both substances in farms. Fourteen E. coli isolates were resistant to apramycin (indicated in sensitive E. Coli and Salmonella sp. isolates in swine), despite the fact that the consumption of this agent was
not observed in the official registers, which might have resulted from the administration of medicated feed or from off-label use. Medicated feed may have come from anywhere in the European Economic Area because there are similar authorised premixed medicated feed in Portugal, as a pre-requisit. However, regarding gentamicin, while no consumption was identified, 6 *E. coli* isolates were also resistant to this agent; however, consumption in this case might have been an off-label use or illegal use of medicated feed because there are no medicated premixes containing gentamicin available in the Portuguese market that would allow for the national production or intracommunity trade of this medicated feed. Tylosin was not tested; however, it was frequently consumed by pigs, while resistance to neomycin was detected in 2 *E. coli* isolates only. Eleven *E. coli* isolates were resistant to enrofloxacin, and 8 *E. coli* isolates were resistant to colistin; these agents were the seventh and the sixth most frequently used veterinary antimicrobials in piglets, respectively. Ceftiofur was consumed by piglets, and despite the fact that cephalosporins have been known to exert a high selective pressure, no *E. coli* isolates were detected as resistant, 1 isolate was intermediately resistant and the 5 other isolates were sensitive to cephalosporins. Ten and 3 *E. coli* isolates resistant to the fluoroquinolones, flumequine and marbofloxacin were identified, respectively, and although flumequine consumption was not reported in this population, marbofloxacin was administered to fattening pigs; this result was not compatible with the obtained resistance data, suggesting potential deviations from the legal use of VAB in general and off-label use and/or medicated feed administration in particular. Cross-resistance phenomenon is not excluded. Intermediately resistant isolates were seldom identified. However, sensitivity was most frequently identified towards gentamicin, colistin and enrofloxacin; all these antibiotics, including the human enrofloxacin main metabolite, ciprofloxacin, were also consumed by men in LVTR in 2013. Meanwhile, colistin has been classified as a critically important antibiotic
for human use [89] and, as such, has been reserved for use in the treatment of serious infections due to resistant bacteria. The *E. coli* isolates studied were, however, of animal origin and exhibited sensitivity to all of those antibiotics, including quinolones and cephalosporins. It is, therefore, essential to gather robust data on animal veterinary antimicrobials consumption to avoid any misinterpretations of the resistance monitoring results and to build a robust and integrated surveillance system to fight AMR [1,9,114,120,175].

LVTR is inhabited by approximately 1/3 of the country’s population and has been through some hydric resource degradation directly associated with the existence and location of anthropogenic polluting activities, including wastewater treatment stations, collective septic tanks and direct discharges [148]. Emergent biological and chemical threats due to pharmaceutical residues in wastewater have been assessed using analytical sanitary surveillance but not in relation to antibiotic use. Twenty-two hospital units [148] and the largest concentration of all the national pig farms are located in LVTR; however, these pig farms have adequate capacity to store their effluents and ensure the required balance between its production, use and destination [150].

5. Conclusions

Outcomes derived using non-harmonized population factors for the assignment of veterinary technical units of measurement for veterinary antimicrobial medicines consumption may be associated with considerable discrepancies in country-specific veterinary antimicrobials consumption profiles, which may then cause misleading interpretations of evaluation results and affect the transparency required to report and publish these results. The criteria required for a sample to be considered sufficiently representative to test a collection model of antibiotic consumption in swine also need to
be refined. Piglets are key factors in swine production and the most critical subpopulation in this sample due to significant differences in in their consumption profiles depending on the method used to calculate their number and their permanence within the herd. Reporting veterinary antimicrobials consumption data may therefore be very controversial and require assignment of a convergent methodology to calculate the number of animals in national populations by species and by production stage; through the determination of this methodology, it may be possible to define a clearer model for data collection and consensual formulas to calculate the animal population, thereby increasing the accuracy of results. Determining the occurrence of resistance to antimicrobials in *E. coli* provided useful data for the surveillance of selective pressure exerted by the use of antimicrobials on intestinal bacteria in food-producing animals. Antibiotic consumption surveillance needs to be complemented with resistance data for bacteria including but not necessarily restricted to zoonotic species with well-known impact; while these data have been substantially reported and studied, other bacteria that are becoming increasingly frequent and resistant, such as *E. coli*, may also be associated with a serious increased need for antibiotic treatment in humans and animals.

It is necessary to further investigate thereafter any possible or potential AMR epidemiological links between men, animals and the environment [6], as this information may disclose actions preventing AMR to be more efficient and alleviate, whenever possible, the pressure on the prescribers of human and veterinary medicines, whose first commitments are to human and animal patients, respectively [126]. The environment is considered to be one of the most important factors influencing population health. However, the concept of environmental health as a consequence of interactions between human populations and the establishment of the environmental risk factors does not cover
particular consideration to animal populations [230,231]. Ensuring the accuracy of veterinary antimicrobials consumption data is crucial [186] and should be aimed mostly.
CHAPTER III

FACTORS INFLUENCING ANTIMICROBIAL CONSUMPTION DATA

COLLECTION

Trends in the Consumption of Veterinary Antimicrobial Agents in Pigs

Abstract

Antimicrobial usage in pigs depends on each production system and on the animal’s different life cycles. One of the main goals of the antimicrobial consumption monitoring in animals, is to reduce it to reasonable levels, and to allow data comparisons among animal species, among countries or regions and between humans and animals. It should therefore generate the most accurate and reliable data upon which some international agreements must be settled, such as the establishment of adequate technical units of measurement for veterinary antimicrobial consumption. These units are being calculated by animal species, on the basis of the quantity of the veterinary antimicrobial medicines that were sold within a certain period of time, and administered to an animal population sample, per life cycle, as indicated in RCMs regarding the posology and the treatment course, after which the amounts are extrapolated to the national pig population, to estimate the total sales, per life cycle.

Pig herds are influenced by many variable factors such as the birth, morbidity, mortality and replacement rates, which sway the farm’s production and permits to manage it, predicting the total pig population in farms, segregated by the different life-cycles, according to the animals ‘weight. These factors are close related with the antimicrobial consumption in farms and are not reflected in the animal census of the national or Community data bases. In the total farms, the number of born piglets that is the most
fragile and shifting pig´s subpopulation, is very difficult to estimate, without adoption of a harmonized average of piglets per litter, whereas the number of the fattening pig´s is more predictive as it depends on the number of piglets to be reared, and the sows are in fact the most stable subpopulation, depending essentially on the replacement rate. Harmonization is however of paramount importance to report national data on animal antimicrobial consumption. This paper reviews some pig productive factors and traits that could be considered to calculate the national´s annual pig population in cross-sectional studies and addresses an alternative methodology to monitor antimicrobial consumption trends in pigs.

1. Introduction
The European Union (EU) produce around 20% of the world´s pig production. In animal production, the offspring is a determinant factor for the farms efficiency and sustainability. Variables, like the number of the weaned piglets per sow, the rates of prolificacy and of fertility are used to inform approximately about a herd´s efficiency. Sow´s pregnancies last 115 days and litter´s size have increased significantly [284,285] in consequence of genetic selection that has been permanently developed in swine, despite the increasing occurrence of peri- and post-natal mortality. Piglets are therefore the most fluctuate pig subpopulation, to be weaned and finished or to be sold. Post weaning and pre-fattening phases is where heavier antimicrobial consumptions tends to occur, due to the animals most fragile phase [232,233,234,235,236,237] conjugating youth with the stress related to the weaning period, movements and clusters.
The animal flow throughout the distinct areas of the farms is usually regular, under a certain productive pattern, which is based on the number of the mated sows, by artificial insemination only, and [238,239] pregnant or in lactation 90% of their lives. The Figure 16 illustrates the productive tree in farms, with a branch with maternity data and another one related to the gestation area, although both branch’s management influence each other results. The average of the piglets born alive per sow (PBAS), is more important than the birth rates [240,241,242,243] and additionally, an increased longevity in sows have also increased the PBA [242].

The piglet’s production is obtained by the monthly average of weaned piglets per sow (WPS= N/A) or by the average of weaned piglets per litter (WPL= N/(A-B+C), where N is the total number of the weaned piglets, A is the number of the weaned sows, B is the number of the weaned sows that weaned no piglets and C is the number of the adoptive mothers used, and the results can diverge [244].

Piglets should be weaned at a minimum of 7 Kg. The smallest (±25% of the litters) are sent to specific areas, for about 1 week, to weight 12 Kg and be sold, while the remaining
animals proceed to the post weaning areas for about 4 weeks, until they weight 25 kg, and go then to the pre-fattening, fattening and finishing areas. At the pre-fattening, clusters are separated by gender, and maintained for about 7 weeks until they weight 60 kg and continue to the fattening sector, for about 8 weeks, until they weight 90-100 Kg and be slaughtered. This weight flow is summarised in Table 14.

Table 14 – Pig productive scheme

<table>
<thead>
<tr>
<th>Pig</th>
<th>Life span (days)</th>
<th>Weight range (Kg/BW)</th>
<th>Final weight (Kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lactating Piglets</td>
<td>1–28</td>
<td>1–8</td>
<td>7–9</td>
</tr>
<tr>
<td>Weaned Piglets</td>
<td>28–60</td>
<td></td>
<td>8–25</td>
</tr>
<tr>
<td>Pre-Fattening</td>
<td>60–110</td>
<td></td>
<td>25–60</td>
</tr>
<tr>
<td>Fattening</td>
<td>110–165</td>
<td>60 to 95–100</td>
<td></td>
</tr>
</tbody>
</table>

Currently, pork leaner meat is often preferred by many consumers [245], and fattening pigs are sent to the slaughterhouses at earlier stages of their lives, which is not however, the case of the Alentejano pig, an autochthonous breed from the south of Portugal, with specific traits [246], and highly appreciated by a specific consumer´s fringe, particularly in the Iberian Peninsula, where prices are above the average pork prices. The average weight of the Alentejano adults [247] is 140-170 Kg in females and 180-190 Kg in males. In the traditional production system sow’s productive life lasts 6-8 parities, while males live for 2-3 years. This breed is not highly prolific (7-9 piglets per litter); [247,248,249,250] piglets are lighter than those from regular breeds and though predisposed to higher post-natal mortality and potentially, higher antimicrobial consumption, to try to invert the tendency. The Alentejano piglets meet is not consumed
as such, and therefore piglets are nursed by the sow from birth (1.2-1.5 Kg) and weaned with 28 days old (6-7 kg) or 56 days old (13-15 kg), depending on the installed production system. In any case, from weaning until piglets are 90 days old (23-25 Kg), they are usually fed in confinement to be then finished in extensive environment. These specificities of the Alentejano pig, which production may have considerable expression, are quite different from the conventional pig characteristics that may also influence the antimicrobial treatments and the amounts to be reported with the technical units of measurement assigned by the ESVAC, which will be necessarily higher [229].

A Portuguese cross sectional study has tested the ESVAC project on monitoring antimicrobial data consumption in conventional pig farms, and particular difficulties were posed to the estimate of the number of the national piglet’s subpopulation, because there are any adopted average for the sow’s litters, (more accurate than birth rates) and piglets may be subject to innumerable movements and treatments among farms. In consequence of the temporary suspension of the ESVAC project [18], no comparative conclusions were however likely to be obtained and there is no predictable date to resume the data consumption collection by species. As for the veterinary antimicrobials sales, the monitoring of the veterinary antimicrobial consumption trends by species, could though be alternatively implemented, for the time being.

An approach to make the best use of the already available consumption data, to assess the antimicrobials consumption trends in pigs, is presented.

### 2. Antimicrobial Consumption Trends in Pigs

The ESVAC model for antimicrobial consumption data collection and monitoring in pigs has been tested [215,217,229] with a sample of 50000 sows in Portugal, (2013), and amounts of veterinary antimicrobials consumed were calculated, separately, for piglets,
fattening pigs and sows. The extrapolated amounts of veterinary antimicrobials (excluding medicated feed) consumed by the national pig population (around three-fold larger than the sample), during that year, was a total of 5.649065901 tons in piglets, 17.43473593 tons in fattening pigs and 0.451062441 tons in sows, shown in table 15, as total amounts of active substance consumption, in the three different pig life cycles.

Table 15 – Tons of active substances consumed, by extrapolation, during the life cycles of pig production in Portugal in 2013.

<table>
<thead>
<tr>
<th></th>
<th>Piglets</th>
<th>Sows</th>
<th>Fattening Pigs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amoxicillin</td>
<td>4.038356491</td>
<td>0.245789312</td>
<td></td>
</tr>
<tr>
<td>Benzylpenicillin</td>
<td></td>
<td>0.017731027</td>
<td></td>
</tr>
<tr>
<td>Cefotiofur</td>
<td>0.084458783</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Colistin</td>
<td>0.643135477</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dihydrostreptomycin</td>
<td></td>
<td>0.059142284</td>
<td></td>
</tr>
<tr>
<td>Doxycycline</td>
<td></td>
<td>2.402976515</td>
<td></td>
</tr>
<tr>
<td>Enrofloxacin</td>
<td>0.430743186</td>
<td>0.226162496</td>
<td></td>
</tr>
<tr>
<td>Flofenicol</td>
<td>0.374109313</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lincomycin</td>
<td></td>
<td>0.012575518</td>
<td>10.77179657</td>
</tr>
<tr>
<td>Marbofloxacin</td>
<td></td>
<td>0.244481658</td>
<td></td>
</tr>
<tr>
<td>Oxytetracycline</td>
<td>0.071258917</td>
<td>0.090673264</td>
<td></td>
</tr>
<tr>
<td>Spectinomycin</td>
<td></td>
<td>0.025151036</td>
<td></td>
</tr>
<tr>
<td>Sulfadimethoxine</td>
<td>0.005836445</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tiamalin</td>
<td></td>
<td>2.066122017</td>
<td></td>
</tr>
<tr>
<td>Trimethoprim</td>
<td>0.001167289</td>
<td></td>
<td>1.723196671</td>
</tr>
<tr>
<td>Tylosin</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

From the analysis of the results obtained, piglets were the major consumers of antibiotics (63%), followed by fattening pigs (32%) and sows (5%). These ratios reflect the weight of the antibiotic dosage in a pig’s population. Figure 17 shows the consumption of antimicrobials distributed by antibiotic class and by pig life cycles (Figure 18). From the
63% antibiotic total consumption by piglets, the majority are penicillins (71.5%), with an identical scenario also observed in sows but surprisingly, in the fattening pig’s population, a lincosamide (lyncomicin) was the most used antibiotic, although a season peak consumption must not be discarded. If such pattern should maintain all over the years, without significant quantitative and/or qualitative deviations, those percentages would stand like consumption references to assess consumption trends, even though transitorily, until ESVAC project may be adequately implemented by all EU-MS.

Figure 17 – Antimicrobial consumption distributed by class in a pig life cycle: (A) piglets, (B) sows and (C) fattening pig. Data collected in Portugal in 2013.
Figure 18 – Antimicrobials administrated to the studied pigs population. The colour scheme follows the antibiotic consumption represented in Figure 17.

3. Discussion

In 2005, the European Commission specifically elected the pig’s production systems to be enhanced with animal welfare criteria, (prohibition of sow stalls and tethering of sows; addressing hunger in sows; prohibition of fully slatted floors for sows; provision of enrichment materials; prohibition of routine tail-docking; Prohibition of routine teeth clipping and grinding; addressing castration of pigs and early weaning) [237] and ever since many developments have been achieved, contributing also to the antimicrobial consumption de-escalation. Animal welfare is an increasingly important factor for animal production and the animal’s living conditions, regarding in particular the free-range systems and outdoor access [251] became a recurrent request by the animal’s welfare defenders despite the discussible public health benefits litters [251,252,253,254], it may represent. The absence of building barriers and the restrictions on prophylatic treatments, may significantly contribute to the increase and spread of infectious agents
Nevertheless, animal welfare perceptions, food quality and safety concepts usually differ amongst the EU-MS consumers, influencing somehow their own national production systems [256]. In some countries, for instance, 40 days is the minimum age appointed to wean the piglets, [253,254] in order to reduce the infection pressure [253] and the inherent antimicrobial consumption. It also reflects cultural aspects and organoleptic preferences.

With the current genetic lines used on pig production, there are also growing concerns regarding the yearly number of each sow deliveries and their unbalanced size litters [257,258,259,260] which number (PBA) has increased from less than 10.9 up to 12.2 per litter, between 1992 and 2001 [246,261] while the mortality rates still varies significantly among herds, [262] with PBA mortality rates from 5 to 7% [263]. Removal rates in pig herds are commonly between 40 and 55% annually [264], which usually relates to 3-4 births at removal.

Regarding the farm’s populations, there is a large variation of reproductive performance between herds [257,265,266] and often a huge number of animal movements, in and out the farms, in leasing or purchasing systems; from “central herds” to other “satellite herds”, functioning as suppliers of pre-pubertal sows, pregnant sows [267] or piglets to be reared and fattened or to reared and sold. The existing close cycle farms, (where antimicrobial consumption data is easier to be collected), may not be the uppermost representative production systems in some countries. In different farms, regions or countries, several or no degree of animal segregation may be actually possible [267,268].

The information from European or national data bases about animal production figures in the different EU-MS, may never be obtained in real time to assist appropriate monitoring. The accurate calculation of the national pig antimicrobial consumption is only possible with the support of the electronic prescription, whereas the data extrapolation from a
randomized animal sample to the animal national population, could be facilitated, particularly in piglets by calculating it, using an harmonized and adopted average of piglets per litter, just like the average animal’s weight by life cycles is used to estimate the overall consumptions.

To calculate the national piglet’s subpopulation, which have a considerable “life turnover” in farms and between farms, and depending on the selected methodology, the results will be considerably distinct not favouring the ultimate goals of the ESVAC project.

However, regarding the monitoring of the antimicrobials consumption by species and just for the time being, while the ESVAC project cannot be fully implemented, and considering its urgent need, to be adequately integrated with the antimicrobial resistance surveillance system, an alternative approach could be the determination of a mathematical equation to estimate the national pig population within a cross sectional study, by extrapolation, from an animal sample with a minimum required number of animals and not farms, as it has been tested in the ESVAC pig pilot phase.

It was expectable that the ESVAC harmonized data collection model, when tested by the different EU-MS, could pose some difficulties but the outcomes to be published may consequently be controversial, advising further reflection and refinement of the model.

Another minor issues that may jump up from the interpretation of results on antimicrobial consumption by the various MS are related either with the pig male breeders kept for regular semen collection and excluded from the pig population, without a rationale for it. Or the pig autochthonous breeds which are significantly out of the conventional pig’s productive weights. The discrepancies between the records on the medicated premixes sales and the official registers in farms of the medicated feed administered to the animals that may occur in several EU-MS, should also be considered. When implementing the
veterinary electronic prescription for the veterinary medicines, the system will have to be extensive to the medicated feed prescription, and legally consigned in the respective community legal acts.

Reliable data on animal antimicrobial consumption are crucial to understand antimicrobial resistance epidemiological links, to manage it adequately and to preserve the antimicrobial agents as a world patrimony, to protect animal and human health for one health only. Furthermore, economic and political factors are decisive [12] to the best success of the ESVAC project.

5. Conclusion

From a food security perspective, and in the basis of a controlled check of veterinary antimicrobials consumption, by oral and injectable administration, not covering other antibiotic containing formulations, it was concluded that the “less harmful” penicillin dominate the control of infections in pig life cycles, although lower quantities of certain antibiotic classes, eventually consumed by lighter animals (piglets) may pose far more risk to AMR issues than higher quantities of some other classes, consumed by heavier animals (sows). For this control’s accuracy, the average of piglet’s litters should be fixed for estimative of this pig subpopulation, with particular productive and commercial traits. The ESVAC project on monitoring veterinary antimicrobials per animal species is crucial for action against AMR but it will take some time to be fully implemented, because overall data collection is complex and requires appropriate legal basis and adequate human and financial resources, still not accessible by all EU-MS. Knowing the annual percentages of different veterinary antimicrobials consumed by pigs, respectively allocated by the different life cycles, it is possible however to evaluate a trend and to identify outlier uses, that should be the exception to the pattern and therefore justifiable
by animal or public health issues, like diseases outbreaks or threats. It is important to ensure that medicated premixes will be part of this consumption monitoring, not only because medicated feed represent a significant (if not the highest) percentage of animal treatments in livestock but also because, when assessing medicated feed prescriptions it becomes very clear that they mostly follow certain patterns of antimicrobial’s use in the same farms, except for particular health reasons or in the course of any disease outbreak.
CHAPTER IV

THE QUALITY OF THE VETERINARY ANTIMICROBIAL MEDICINES

Oral Veterinary Formulations Quality Programmes

Abstract

The aim of this review is to summarise available data on quality of veterinary antimicrobial medicines, on quality programmes and relate it with the oral formulations and dosage regimens, used in the treatments of food producing animals that may have any impact on the antimicrobial resistance (AMR) development. The selective pressure exerted by the antimicrobials’ use in these animals is connected with the increasing emergence of AMR pathogens, and requires efficient mitigation approaches, besides the reduction of the veterinary antimicrobials consumption [2]. Restraining the prophylactic use of antimicrobial agents in animals, it may increase the health risks of bacterial infections, affecting animal’s health and animal’s production, and entailing additional antimicrobial treatments [117]. The development and spread of resistant microorganisms are influenced by the bacteria type, the environment, and/or the veterinary antimicrobial medicines that are administered to the animals, regarding in particular the different formulations, dosages, and routes of administration which efficiency is ensured by quality parameters that should be controlled periodically. Quality incompliances regarding active substances may affect the medicine’s efficacy, by not guaranteeing the adequate concentrations at the locals where it is supposed to exert an action if under-dosed. In case of overdose, safety is also compromised by interfering with the withdrawal periods and/or animals’ toxicity. In any case, AMR selection pressure by distinct mechanisms is also a related risk factor to be seriously considered.
All these factors are linked but very complex to identify in farm conditions.

1. Introduction

The use of antimicrobial agents naturally induces an increasing rate of resistance in bacteria, of human and animal origin. Resistance development can be affected by the veterinary antimicrobial medicines’ dosing regimens because each of them has its own individual characteristics, regarding the distribution in the organism and in the environment, not easing the attempts to minimize the resistance mechanism. In addition, the veterinary antimicrobial concentrations at the site of the infection are also variable [269] and often unpredictable.

In food producing animals, oral formulations (liquids, powders and medicated premixes) are a considerable fraction of the veterinary antimicrobials sales and consumption, almost certainly. The medicated feed is even a veterinary medicines exclusive presentation for oral administration to food producing animals, using animal feed as a vehicle.

Antimicrobial administration routes are also critical to AMR, [270] particularly the oral via, because it is in the gut flora that amplification, dissemination, and circulation of resistant bacteria are significant, affecting both commensals and pathogens, resistant and susceptible, eliminated with the fecal waste as the most important source of the ecosystem contamination. These microorganisms are often related with the human infection with foodborne resistant bacteria of animal origin.

During antimicrobial treatments, the concentration’s gradients vary, depending on the rates of the drug’s diffusion and elimination, generating different selective pressures. A subpopulation of resistant bacteria that often exists may be selected by those concentrations, leading to a regrowth during treatment [271]. Low antibiotic concentrations can select for low-level resistance, which can have a major effect on the
emergence of high-level antibiotic resistance [272]. Differentiation between susceptibility and resistance of bacteria to antimicrobials is commonly based on microbiological criteria but the distribution ranges of both strains may overlap, and the only way to determine susceptibility is to search for acquired resistance mechanisms or resistance determining genes in these strains, if those are known.

A simple method to measure resistance and detect changes in susceptibility is the MIC (Minimum Inhibition Concentration) which does not detect minor resistant subpopulations. In contrast, the Mutant Prevention Concentration (MPC) is the lowest antibiotic concentration that prevents growth of the least susceptible single-step mutants in a population but any treatment dosages that yield concentrations above the MPC during the whole dosing interval, would pose pharmacokinetic and/or toxicity related limitations. Dosage regimens needed for eradication of the main bacterial population may be associated with a risk of selecting preexisting or newly formed mutants and in these cases, higher dosages may be needed to slow down resistance development. Due to suboptimal dosing regimens or quality incompliances related with the active substances concentration of the veterinary antimicrobial medicines, target bacteria may though be only weakly inhibited, despite the usual collateral effect on the microbiota that antimicrobials often have, altering its composition, during long-term treatments in particular [273].

The treatment of all clinically healthy (but presumably infected) animals within the same group of animals with clinical signs of a contagious disease is known by “Metaphylaxis”, using specific veterinary medicine’s formulations [86]. When antimicrobial veterinary medicines are used metaphylactically, (usually with oral formulations), some animals may be administered subtherapeutic doses and in consequence, the progressive selection of antibiotic resistance will differ from that expected at lethal concentrations, where only rare pre-existing mutants with high-level resistance, usually survive [272]. To achieve the
same effective antimicrobial concentration in the target sites, higher doses are needed for oral therapy than for injection and in food producing animals, antibiotics are administered both ways, except on certain species or over certain productive life cycles, where treatments are exclusively via feed or drinking water.

The metaphylactic treatments justify when any infections outbreaks may occur in a herd/unit due to the introduction and quick spread of a certain microbe that rapidly causes clinical disease in a large proportion of the animals or when an external factor causes secondary infections by opportunistic bacteria harbored in the animals or within the herd.

For highly contagious and/or severe diseases simultaneous treatment of clinically diseased animals and metaphylaxis of clinically healthy animals that are likely to be in the incubation phase (due to close contact with diseased animals or exposed to the same external factor) may be justified from an epidemiological point of view. The objective would be to control disease spread and/or prevent further development of clinical signs in the group. However, metaphylaxis is an easy way to treat a large number of animals at the same time, and it can be used for prophylactic treatments or for “growth promotion shots” in very specific periods of the animals´ production, enough to avoid unwanted productive losses.

The metaphylactic treatment according to the proportion of the clinically diseased animals and the severity of the clinical signs should thus be always justified on epidemiological and clinical grounds.

Some oral formulations like the products to be diluted in the drinking water or mixed with feed, allow only a claim for both treatment and metaphylaxis because all animals will be treated independent of their individual clinical status [86].
2. The oral forms of the veterinary antimicrobial medicines

The veterinary antimicrobial formulations, with one or more active substances, are developed to be used in one or several animal species with different physiological and metabolic profiles. In food producing animals, oral forms are mostly administered via drinking water or feed (blending, top-dressing and medicated feed). The advantage of medicating through drinking water is that unhealthy animals usually drink more than they eat, and since animals drink in general, twice more water than they consume feed, the concentration of the drug in the water needs to be approximately half of the concentration that would be needed in feed, although rigorous individual dosing is always impossible to estimate.

Medicated feed manufacture is a simple mixing process from one or more authorised medicated pre-mixes, in feed mills or farms, where manufacturers are responsible for its quality parameters control, ensuring that it is a homogeneous and stable mix without any undesirable interactions and cross-contaminations. The medicated feed should also undergo regular official checks, and laboratory tests, to ensure compliance regarding homogeneity, stability and storability, at all stages of its production and shelf-life.

The medicated pre-mixes are veterinary medicines that after incorporation into feed, produce a medicated feed, which is no longer regulated as a veterinary medicine, but by another distinct legal act [21,22,274] and having thereof more flexible requirements for good manufacture and good distribution practices.

The ESVAC (European Surveillance of Veterinary Antimicrobials Consumption) 5th report clearly shows (Figure 19) that medicated pre-mixes are a major sale, followed by oral powders for top-dressing animal´s daily rations. Medicated feed is often provided to food producing animals ad libitum, like regular feed, which is necessarily associated with imprecise intake of the active substances’ concentrations, leading to over administration
with risk of animal toxicity and food residues or under administration that may lead to treatment failure or the selection pressure of AMR microorganisms.

Figure 19 – Distribution of sales of veterinary antimicrobial medicines agents for food-producing animals (mg/PCU), by pharmaceutical form, by country, for 2013. (ESVAC 5th Report)

Consumption profiles of medicated premixes and oral powders in the different MS (figure 20) can be influenced by the commercial availability of authorised medicines at national level and by national policies regarding feed medication legislation despite the ruling Community directive governing the medicated feed, due to a substantial lack of harmonization on its transposition in the different EU-MS, and currently under the ongoing Community.

Figure 20 – Oral solutions, oral powders and premixes as percentages of total sales, (mg/PCU), of veterinary antimicrobial agents for food-producing animals by country, for 2013. (ESVAC 5th Report)
The animals feeding with sub-therapeutic doses of antimicrobials as growth promoters over long periods of time was banned in the European Union, which represented a significant reduction in selective pressure for antimicrobial resistance traits but judicious use of medicated feed and oral powders and liquids [20], to avoid replacement of growth promoting action, has to be ensured [116].

Antimicrobials are the most cost-effective way to maintain or improve the health of animals and the continuous use of antimicrobials in feed may conduct to the overuse and misuse of antimicrobials in food producing animals, particularly to enhance feed efficiency. Additionally, medicating via feed or water is not an efficient way to achieve accurate serum and plasma concentrations which is of particular concern regarding the antimicrobial substances, because the animals most in need, may be the ones least likely or able to access the necessary dosages to be treated, and that may drive selection for resistant microorganisms.

After oral administration, several factors like feed nutrients, commensal bacteria and organic diseases may influence the absorption and the pharmacokinetics of an antimicrobial agent and prevent it to get the target microorganisms with the most appropriate dose. Some of these factors are related with the determining target organ dose.

Overall, the administration of antimicrobials to animals for reasons other than treatment, poses an unnecessary public health risk, contributing to a considerable fraction of antimicrobial-resistant infections.

3. Quality control of the veterinary antimicrobial medicines

Unpredictable dosage outcomes may result in consequence of undetected quality defects, of veterinary antimicrobial medicines administered to animals due to eventual combination of the active substances with feed ingredients or due to cross-contamination
events. The medicines quality system insurance should check the quality of the finished product in all parts of the distribution chain as described in the medicine’s quality file submitted to grant a marketing authorisation.

The quality controls of the veterinary medicines are mandatory and it is performed at the Community level (EMA) for the centrally authorised products and at the national level for others. No results are published and only the marketing authorisation holders and the national competent authorities are informed about any detected incompliances. There are reasons to believe that the national control programmes are not performed regularly, because it is expensive, it is not required by the EC /EMA and may be neglected for being considered a non-safety issue. This gap may however be significant to AMR emergence.

The sampling plan of the medicines quality control system is annually established on risk criteria related with the active substance *per se*, the manufacturer process, the route of administration, the target population and eventually with a submitted variation to an already granted marketing authorisation. To establish such risk criteria, information is collected from the EU-MS, regarding the number of the sold packages, specifying those medicines with reduced stability, that are usually administered at low dosages, with narrow therapeutic windows, indicated for long treatment duration, with manufacturer or formulation complexity and with potential toxicological impurities. New generic or recent innovator medicines and recent variations or specifications regarding manufacturer that may have been submitted, are also taken into account.

When the national competent authorities perform these programmes, it’s not unusual to detect non compliances mostly associated with critical parameters such as the dissolution assays and the active substances dosage. It was no possible to get any veterinary information on national quality programmes. Results should however and in any case be published due to is particular importance regarding antimicrobials.
A Community sampling and testing programme has been running in the EEA (European Economic Area), since 1997 for the centrally authorised medicines, 3 year after their marketing to ensure that the respective quality control methods are satisfactory as proposed in the marketing authorisation dossier and to investigate any suspected quality defects [275], or to detect/confirm counterfeits. The selection criteria are risk-based as described, and include risks that might have been identified in relation to active substance but not for specific pharmacological classes of medicines, such as antimicrobials. The patient profiles, the product’s poor stability, the production process, the pharmaceutical form and any relevant data from previous controls are often factors that are taken into account for sampling and testing. Particular consideration is given to inherent variability of the production processes, regarding the product’s poor stability, the potential presence of toxic impurities, and to problematic bioavailability, and biological standardisation of the potency. Human and veterinary medicine’s selection for annual control [82,83] is similar not considering specific factors that may affect veterinary medicines only or specific formulations like medicated premixes, oral powders to mix with feed or dilute in the drinking water, teat dips, spot-on and the multispecies presentations, are for instance, veterinary specific.

The first Community programme was carried out between 1998 and 1999, and only 9 products that were elected, according with the respective therapeutic categories, their market availability, and the stability and the manufacturing process of those products. These and other factors were thereafter addressed under a “risk-based” approach to assess the human and veterinary medicines against defined risk factors that consider both the probability of an adverse outcome from the testing, regarding the testing itself and/or the testing results, and the possible consequences of this outcome in relation to the severity of the impact. Reports, relates only to numbers of medicines tested, (Table 16). The risk
level provided is defined as a health risk and only national authorities and marketing
authorisation holders do know which medicines have been tested.

Between 1998 and 2000, half of the 45 tested products, had identified some issues. [276]
Between 1998 and 2007, a total of 280 products were tested.

Table 16 - Results of the Sampling and Testing Programm (EMA reports)

<table>
<thead>
<tr>
<th>YEAR</th>
<th>PRODUCTS TESTED</th>
<th>NO PROBLEMS IDENTIFIED</th>
<th>ISSUES IDENTIFIED</th>
<th>OUT OF SPECIFICATION</th>
<th>OUT OF SPECIFICATION (HEALTH RISK)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2008</td>
<td>6</td>
<td>2</td>
<td>4</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2009</td>
<td>8</td>
<td>5</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>2010</td>
<td>8</td>
<td>2</td>
<td>4</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>2011</td>
<td>8</td>
<td>3</td>
<td>3</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>2012</td>
<td>8</td>
<td>3</td>
<td>4</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>2013</td>
<td>7</td>
<td>3</td>
<td>3</td>
<td>1</td>
<td>-</td>
</tr>
</tbody>
</table>

In 2008, the products tested were in compliance with the authorised specifications and
there were no instances of quality defects raising immediate concern for public or animal
health [277] but in 2009, one “out of specification” product raised concern for animal
health and all the batches were recalled beside the suspension of the respective marketing
authorisation [278]. The products reported to be “out of specification” in 2010 were
further assessed and submitted to variations to meet compliance [279] while in 2011 the
results showed four products outside their specifications, and one veterinary product in
2012 [280,281]. Most of the products tested in the 2013 programme were in compliance
with the authorised specifications, except one veterinary product [282]. These results
clearly show that the probability of quality incomiances in the veterinary medicines is
considerably high, considering the limited samples that were tested and involving
centrally authorised products only, which ensure the highest level of harmonization in the marketing authorisation procedure.

3. Discussion and Conclusions

There is still a lack of the optimal dosing regimens to treat bacterial infections preventing simultaneously the selection and emergence of resistance. Difficulties in ensuring precision in antimicrobial dosage, fails to deliver predictable, uniform, or intended dose levels. The administration of antimicrobials may select or co-select for AMR microorganisms and determinants in the commensal or pathogenic bacterial populations in animals. Co-selective and cross-selective pressure make resistant strains eradication rather complex [114]. When medicines are administered orally to animals via feed or drinking water or when the quality of those medicines or medicated feed is defective, eventually combined with other factors that may influence the distribution and the pharmacokinetics of the veterinary antimicrobial medicines in the organisms, health risks may raise. The oral route administration anticipates the most direct impact of the antimicrobial agents on the animal’s digestive tract, particularly on the gut as the largest organic reservoir of commensal bacteria and pathogens, both resistant and susceptible and though be regarded as the most promoting via to select pressure on AMR. On the other hand, it is the most practical way to administer medicines to animals. The risks of overdosing, with higher probability of residues occurrence or under dosing are posed, not treating diseased animals, and/or promoting AMR.

When injectable forms are not an option, water seems to be a more appropriated antimicrobial vehicle, rather than feed, because only half dosage is needed. This may be in line with the need of de-escalation of the antimicrobials use in animals but it may have impact on AMR selection pressure, both in healthy and in diseased animals, beside
sometimes dosage strategies may require higher dosages and shorter treatments. The concept of achieving MPCs would increase antimicrobial exposure of commensal flora and raise total antimicrobial consumption which is associated with increasing resistance. The inevitable presence of veterinary medicines’ residues in food, even at concentrations lower than the MRLs fixed, may have any significant effect in altered human gut flora and even though concur to the AMR phenomenon, as it is not determined whether or not those metabolites (or parent compounds most of the times) are chemically changed by cooking or in the course of food technological operations, into molecules without any antimicrobial action. The studies to evaluate the impact of veterinary antimicrobial residues, in human gut flora, below the MRLs cannot predict any concomitant human treatments with antimicrobial substances for human use. The amplification and spread of resistant bacteria is generally caused by antimicrobials or their residues in the reservoir [114].

Health consequences of exposures to antimicrobials and its metabolites at environmentally relevant concentrations also remain uncharacterized. Although problems arising from the veterinary antimicrobial medicines quality aspects may result in inaccurate dosages that favours resistance emergence, antimicrobials are not systematically included in the European quality testing programme as they are not considered major safety concerns. Information on national programmes were not available. For the establishment of the “risk levels” to observe in the centralized medicines quality testing samples it is considered that all medicines are in principle, GMP produced and rigorously controlled and that the conventional risk ranking process (severity factors vs the probability of occurrence) is not used, it may seem inappropriate to suggest that certain products are more likely than others to result in an adverse testing outcomes.
Medicated feed is however not part of this programme and medicated pre-mixes GMP are specific and more flexible than for any other veterinary medicine’s GMP.

The quality control of medicated feed is also regulated in the Community, and it is the manufacturers that are responsible for monitoring but apparently for internal use only, considering that no results are ever known or published. These manufacturers are often the farmers who own the animals to which the manufactured medicated feed is administered (auto-producers).

Moreover, the medicines’ good distribution practices also doesn’t cover medicated feed, which under certain conditions of storage and conservation, particularly over long distance transportation, may have deleterious impacts in the quality. With a crescent number of centrally authorised medicated pre-mixes, and a diminishing number of medicated feed manufacturers, free intra-Community trade of medicated feed may also increase.

As a global concern [120], results from both European and national’s quality testing programmes should be published and competent national authorities should be encouraged to reinforce systematic sampling of antimicrobial medicines and medicated feed, to be also part of the integrated veterinary surveillance system on AMR and on antimicrobial consumption.
CHAPTER V

DISCUSSION

This thesis typifies the use of the veterinary antimicrobial medicines in the frame of the European regulatory system, to protect animal health and to ensure food security.

The preamble to the public health role of the veterinary medicines and the need of the veterinary antimicrobial consumption monitoring is essential to understand the magnitude of adequate veterinary surveillance systems, capable to integrate related data from other monitoring programmes on animal diseases (including zoonosis), on residues, on the medicine’s quality (including the medicated feed), on resistant bacteria of animal origin and even on integron content in \( E.\ coli \) and \( Salmonella \) populations to elucidate the evolutionary changes of gene cassettes, which may be fundamental in estimating the health risk and preventing the spread of particular antibiotic resistance determinants from animals to humans [174,283,284,285,286,287,288,289].

Environmental data are critical to complement integrated surveillance systems of such nature, contributing to further investigation on resistance epidemiological links. Grave et al., (2006) [163] suggested that the monitoring of the antimicrobial treatments in animal populations, at regional or national level, would also provide an enhanced basis for the residues monitoring programmes [79] evidencing interfaces and intersections that may concur for health and safety guarantees [290,291].

It was considered particularly relevant to highlight the veterinary medicines’ specificities, beyond the animal health sphere to expand communication and prompt inter sectorial involvement, in line with the One Health perspective [292].

Medicinal products are distinctly used in companion animals and in food producing animals, due to the different roles both type of animals have in societies [293]; when
raised for human consumption, additional concerns are also raised and despite the
crescent demand on animal protein, the intensive animal production systems are
permanently being argued, and regularly accused of considerable amounts of antibiotic
consumption in farms, although empirically most of the times. Such estimates are in
principle about to be replaced by real antimicrobial consumption measurement and report
[294,295,296].

At the present time, the world is being asked to react to the increasing number of multi-
resistant microorganisms by containing the AMR phenomenon that needs to be tackled
holistically throughout all the society quadrants sustained by sound scientific grounds
[297,298].

For effective action against AMR, better information and communication are essential for
the best management possible of the AMR, relying on appropriate surveillance systems
that may disclose in time, the most useful data to protect animal and people [299].

Although the surveillance systems on human antimicrobial consumption are relatively
recent (the ECDC was established in 2004), [284] the veterinary sector progressed on the
same direction just with a five years delay, and all the efforts are ongoing to go along
with the human achievements. The first jointly report from EFSA/EMA/ECDC has
already been published (2015) [155].

The need of accurate data to feed effective surveillance systems on animal antimicrobial
consumption is critical and urge further refinement of the ESVAC project. The available
information on sales of the veterinary antimicrobial medicines in the EU is, at least in
parts and regarding the medicated feed in particular, incomplete and inconsistent [75]. As
this information is the basis of the antimicrobial consumption monitoring by animal
species, and thereafter used to assign the veterinary technical units of measurement,
additional care must be granted to guarantee accuracy and sustainability of the veterinary surveillance project [300], with paramount importance for AMR management [298].

It is worthwhile to retain that the production trends of medicated feed in the EU, vary across countries where there is considerable uncertainty about production and consumption because official statistics are rare, preventing the inclusion of the medicated feed in the ESVAC project and inferring that it would be covered by the medicated premixes sales. This option was mistakenly taken because the ESVAC data reflect the overall European sales but not at the EU-MS level, despite the objective to compare regions and countries’ antimicrobial consumption profiles.

The number of the authorised medicated pre-mixes varies significantly across countries mostly based on pre-mixes containing rather old active substances and not subject to innovative markets [75]. Medicated premixes and medicated feed are predominantly debated due to the correlation with massive animal treatments and metaphylaxy.

Nowadays, particularly in the northern countries of the EU, the medicated feed tend however to loose importance when compared to other oral ready-to-use formulations to dilute into water or top dressing/blending into feed. These preferences are due to the medicated feed production costs that are significant in countries where production levels are low [75] although such tendency is compensated by the intracommunity trade operations that could and should also be monitored (Fig. 21). Alternative options depend on many factors, like cost advantages, specific VAT rules, monetary incentives, tradition, and effectiveness/efficiency experiences [75]. Price always matters and whether medicated feed is a more costly or a more cost efficient alternative of administering oral veterinary antimicrobials, it depends on the manufacturers, on the active substance used and on the specific EU-MS. All these factors clearly reveal the economic primacy of the in-feed formulations, considered to be equally efficient, but with the exception of the
medicated feed all the other oral presentations are handled to be mixed with the feed in farms posing potential risks of occupational AMR among workers.

Antimicrobial agents are by far, the most important veterinary medicines currently used for the production of medicated feed, which is still a regular way of oral administration to animals in some EU-MS, and for which detailed data should be available, as real consumption. Furthermore, it is not clear whether the medicated feed consumption is interpreted by all EU-MS as consumptions of antimicrobial veterinary medicines, considering that only registers of these are mandatory at the farm level.

Records on veterinary antimicrobial´s administrations in farms are available for monitoring, and are in theory, the most important data source to feed the antimicrobial consumption surveillance system. Medicated premixes are veterinary medicines only administered via medicated feed that should be registered as veterinary medicines, although different interpretations may consider that a medicated feed, which legally is no longer a veterinary medicine, is therefore excluded from the registers obligation. This inconsistency may suggests that a specific provision regarding the registers obligation of the medicated premixes consumed in farms, necessarily via medicated feed, should be explicit in the Community veterinary medicines’ legislation as it is already in the Portuguese legal act. In addition, the registers may be the farmer´s or the veterinarian´s responsibility, depending on whom had administered the medicines to the animals, but the records are farmer´s property [301].

In any case, these formulations cannot be precluded from animal production [20,75] despite it may raise some concerns, because antimicrobials given orally and for long periods of time, are known to facilitate the prevalence of resistant bacterial strains [20,114]. Animal mass medication (with oral liquids, powders or medicated feed) is
however already restricted to situations where it is strictly necessary in accordance with
the veterinary decision and under the SPCs´ terms.
To disclose accurate data, farmers have to be convinced that they are in fact contributing
for a cause and not being inflicted by random exposures without any added value for the
business. Besides, there are still a considerable number of small farms where these
register are rather difficult to monitor and collect, underlining the importance of the
electronic prescription as the second most reliable data source to feed consumption
surveillance systems. Daily work on farms is demanding and both farmers and
veterinarians often complain of additional “administrative burdens” which are key factors
for effective surveillance [188].
The veterinary antimicrobial medicines´ marketing authorisations are granted when their
efficacy, safety and quality are demonstrated and validated. Specific aspects of the
veterinary medicines are thereafter monitored by the competent authorities particularly in
relation with manufacture practices and quality programmes, advertisement, market and
usage rules, pharmacovigilance and residues in food of animal origin.
The use of the veterinary medicines is not an exclusive responsibility of the veterinarians
because there are other professionals entitled to prescribe medicines to animals, including
in some EU-MS, which may conflict with the guarantee and the traceability of the
responsible use of the antimicrobials in animals.
Under the ongoing review of the veterinary medicines Community code, one of the main
proposals is the consignment of the prescription for antimicrobial medicines exclusively
to veterinarians. However, the prescription criteria is already established for the
veterinary medicine´s classification as POM or non-POM and different prescription
rational for different POM don’t seem either to be reasonable. It should be ensured
instead, that all POM are prescribed by veterinarians only, and exclusively supplied by
the authorised channels. The decoupling issue would be interesting to analyse in the context of the retail harmonization in the EU, which is not in the European Commission’s agenda whatsoever.

The control of the public access to the human and veterinary antimicrobial medicines is nevertheless a major issue for regulatory action against abuse and misuse of the antimicrobial medicines.

The human medicine’s prescriptions are fundamentally controlled by the national health systems for prices and reimbursement policies [121,302], whereas the veterinary prescriptions are actually not controlled, and farmers, who are the veterinarian’s employers, often interfere or decide on the veterinary medicines selection. Inappropriate advertisement of veterinary medicines, directly to farmers may have serious public impact, because prices are often the exclusive acquisition determinant factor. The public service rendered by veterinarians, even at private farms, should in principle be exclusively hired by the governments or have at least a minimum contractual relationship with the competent authorities to favour the independency principle.

Under good farming practices the veterinarian’s responsibilities include the prevention, identification and treatment of the diseased animals, using the most appropriated medicines, privileging the efficacy, the route of administration, (influenced by the therapeutic action at the site of infection and the ease of usage), and the indicated withdrawal periods [303]. These opinions are based on own professional experiences [81] but also on the epidemiological profile of the farm, and on TSAs whenever necessary and available. The route of administration is often limited by the animal species to be treated, and by the stress it may cause to animals over treatments.

Farmers with the veterinarian supervision are responsible for the implementation of health and welfare programmes in order to promote animal health and food safety
It is also their responsibility to isolate diseased animals to avoid the transfer of pathogens, to comply with the storage conditions of the antimicrobial medicines in the farm, to promote the adequate hygienic conditions between people and the animals treated, to comply with the recommended withdrawal periods and to manage any leftovers and disposable packages of the medicines used. It may thus be concluded that veterinarians and farmers are major players in the antimicrobial responsible use in food producing animals.

In order to prevent clinical disease and ensure animal welfare, groups of animals that may have been exposed to pathogens, may need to be treated sometimes without an accurate diagnosis. Veterinarians are aware that proper diagnostics and TSAs are valuable tools to enhance the antimicrobials efficiency by restoring the health status and reducing the antimicrobial usage. They are also aware that in-feed medication is used to control a problem and is not a long-term solution to substitute hygiene, biosecurity, feed, vaccination if available, etc. Despite the recognition of the upmost value of the screening tests to detect either the pathogens or its sensitivity to antimicrobials, time and costs are always considered. However, when first-line treatments fail or the disease recur, the second one is often based on TSA results. Sometimes, these results include unavailable veterinary medicines or veterinary substances to be used under the “cascade” legal provision. The accuracy regarding resistance detection is equally important, requiring particular attention on harmonization of the methodology adopted, to ensure fair comparisons [301].

The EU-MS are periodically subject to FVOs (Food Veterinary Officers) audits, to verify the compliance of the NCAs with the Community legal acts, to ensure food safety and food quality, which is scattered by several legal acts, governing veterinary medicines, medicated feed, residues, forbidden substances in animal production, resistance in
zoonotic bacteria, animal welfare, diseases’ eradication programmes, food quality parameters, and the regular activity of distinct sort of establishments, including laboratories, slaughter houses, farms, wholesalers and retailers. Independently of the follow-up measures and/or legal consequences of such audits, the countries’ reports are thereafter published in the internet and consulted all over the world, mainly for animals and foodstuff trade purposes, clearly denoting the economic weight of the animal production for individual countries. Data on AMR and antimicrobial consumption is also frequently solicited by markets, investors and third countries’ NCAs. The CVOs also audit the third countries with special commercial agreements, to export foodstuff of animal origin to the EU, complying with the EU specific legislation as a safety guarantee for the European consumer.

In the EU, there are national and Community active pharmacovigilance systems to monitor the efficacy and the safety of the veterinary medicines in animals and users, although the lack of the antimicrobial’s efficacy due to AMR is not imputable to “the” medicine itself and is therefore out of its scope.

Regarding the veterinary medicines residues in food, the controls are performed in accordance with the EC annual list of molecules to be screened from samples collected at farms and slaughter houses. Although in line with the current European food safety policies and international agreements, this procedure continues to monitor a recurrent set of forbidden substances [292] in detriment of the most sold/consumed ones. These programme’s funding is also becoming increasing constraints because MS often struggle to finance their share and the specific analytical methods are permanently evolving, posing the reference laboratories some fitness challenges that are often questioned. As a consequence, there are many substances that are systematically used in animals but rarely tested for residues. Results have never been available for public consultation.
The withdrawal periods of the veterinary medicines are determined in accordance with the residues depletion studies of the pharmacological active substances, determining the moment when residues concentrations comply with the fixed MRLs, although the time necessary to total residue depletion is unknown and the zero residue criteria is unrealistic to apply on food production [304]. The antimicrobial’s marker residues, that are predominantly the parent compounds, are therefore eliminated at low concentrations for undetermined periods after the withdrawal periods, and potentially exerting continuous pressure on bacteria, in any host, including in the environment, which is not subject to legal limits of antimicrobial substances [305,306].

The only available studies on the impact that the residues may exert on the human gut microorganisms after ingestion of food of animal origin with residues, are those submitted within the marketing authorisation procedure, not performed in humans under or after antimicrobial treatment. Thus, the inevitable presence of residues in food, even at concentrations lower than the fixed MRLs, may have any effect in human gut flora and concur to the AMR phenomenon. It is unknown whether those metabolites are chemically changed by food technological operations (including cooking) into molecules without any antimicrobial action, although it is known how those processes may affect a considerable range of resistant and susceptible microorganisms, ensuring food safety.

For the time being, in the veterinary sector, an HACCP (Hazard Analysis and Critical Control Point) approach throughout the food chain may be effective to control foodborne pathogens [117,307] and thereby reduce foodborne illnesses, because although alternatives to the use of antimicrobials are being already explored, none of them can replace the therapeutic indications of those substances. Thus, to maintain the continued efficacy of the currently available antimicrobial agents, it is critical their most prudent
use, for the shortest period of time possible and respecting the established withdrawal periods, in accordance with the respective SPCs.

Due to the severe veterinary medicines availability issue, the incompatibility with the off-label use, by not respecting the exceptional use provision is fully recognized but there is no alternative to treat some animal species for which no veterinary medicines have been developed in the EU. Moreover, the off-label use is subject to administrative withdrawal periods that sometime exceeds the animal’s productive life and have no safety scientific grounds. The impact on AMR can’t even be risk base approached, because it is also unclear whether the EU_MS have any available records on the off-label use. In any case, it is mostly improbable that the rational for those uses are described on a case by case basis, respecting the “cascade”.

Concerning the veterinary medicine’s quality programmes, there are national controls in the EU-MS that are coordinated by the respective NCAs, and Community control programmes, exclusively for the centrally authorised medicines that are coordinated by the EMA and performed by the EDQM (European Directorate for the Quality of Medicines). However, results are not available for consultation at national or Community level. For the medicated feed, which manufacture process is a simple mixing, it is the manufacturer's responsibility to carry out the quality control of the products before consumption [21,22]. Overall, regarding the quality parameters to be monitored, the NCAs, usually rely either on the MAH controls or on the manufacturer’s auto-controls, and ultimately on the Community annual programmes, which sampling criteria don’t reflect any specific concern about the importance of the quality of the antimicrobial substances. Despite quality defects or incompliances may be linked with the homogeneity and stability of the active substances which may impact on dosages and consequently contribute to influence AMR pressure, the quality control of antimicrobial veterinary
medicines and medicated feed have been rather infrequent or rare, to ensure that the quality and concentration are maintained until the expiry date, under the recommended storage and transport conditions. Medicated feed in particular are often long distance transported and therefore subject to deleterious quality impacts on the product. Formerly, medicated feed were produced under prescription only but nowadays the prescription is required to sell already manufactured medicated feed. In-between storage and transport conditions should prove topo maintain proper concentration and ensure proper dosage. It is known a certain lack of precision in antimicrobial dosage that fails to deliver predictable, uniform, or intended dose levels, when medicines are administered via feed or drinking water but when a quality defect affects the product stability, or when the distribution and pharmacokinetics may be altered after oral route administration, the risks of overdosing or under-dosing are higher. The probability of residues occurrence or the inefficiency of treatments on diseased animals and AMR promotion also increase proportionally.

The administration of veterinary antimicrobials may select or co-select for AMR genes in the commensal or pathogenic bacterial populations in animals and there is still a gap between the optimal dosing strategies to treat bacterial infections, and the simultaneous prevention of AMR selection and emergence [307].

The veterinary antimicrobial substances are frequently the same or closely related to those used in human medicine and in horticulture, all of them competing to the AMR development [20], which is a global public health concern, because the antimicrobial agents are essential medicines for human and animal health, people wellbeing and animal welfare [73,163,308].

In conventional as in organic farms [304], human co-inhabitants or workers, the domestic and wild animals, pets, birds, insects and the environment (water, soil, feeds, wastewaters,
sewage, manure, lagoons, etc.) may be resistance host compartments [111,114]. Nowadays, multiple resistant bacterial strains are being increasingly found in humans, particularly in health care units due to a survival advantage in those environments where multiple antimicrobials are used, whereas in farms, except during unexpected outbreaks, the treatment patterns are usually maintained and often using the same set of old antimicrobial agents, against a “recycled microbiota” [284]. It is important to highlight that the impact of the veterinary antimicrobial use on the emergence and dissemination of resistance traits and its contribution to the antimicrobial resistant pathogens in humans, remains overall poorly quantified and requires further investigation based on accurate data [107,122]. In addition to plasmids, other genetic elements that participate in resistance gene transfer and the consequent development of antimicrobial resistance in bacteria are transposons and integrons [309]. These integrons are capable of capturing and excising gene cassettes, which are natural genetic engineering platforms that encode resistance to several antimicrobial agents, and are frequently associated with the development of multidrug resistance in gram-negative bacteria. Enterobacteriaceae may cause serious infections in humans and animals, and many of the most important bacteria, particularly E.coli and Salmonella, are becoming increasingly resistant to commercially available antibiotics and carry integrons containing resistant gene cassettes. Thus, it is crucial to track the evolution of multidrug-resistant isolates and to analyse the implications for humans. Surveillance of integron content in bacterium populations can provide useful information concerning the evolutionary changes of gene cassettes, which may be fundamental in estimating the health risk and preventing the spread of particular antibiotic resistance determinants from animals to humans [283].

Thus, the prudent use of antimicrobials seems to be for now a main strategy to contain antimicrobial resistance and a key factor for the food residue and microbiological safety.
Although the dissemination and transmission pathways of antimicrobial resistant bacteria are very complex and not fully understood, there is some evidence of a food safety issue of animal origin [123, 284] with potential serious consequences in human health. Bacteria spread across animals is complex and has not been fully investigated, whereas the spread of resistant bacteria from humans to animals, either by direct contact or via the environment, has not been well documented [114], despite the strong evidence of AMR transmission from human sources [121] as well.

Some environmental and animal protection measures are also inconsistent with the AMR concerted fight strategy. The highly defended preservation of certain wild necrophagic birds in the EU for instance, fed with animal carcasses placed in the open fields, completely ignore their potential role on AMR development and spread. Animal welfare is an increasingly important factor for animal production that has contributed substantially for the reduction of the animal’s treatments but increasing demands by animal’s and consumer’s associations on animal’s housing and living conditions, particularly regarding the free-range systems and the outdoor access, may pose discussible public health benefits and AMR increased risks too.

Moreover, there is little or no information about the potential impact on human or animal health from the use of antimicrobials in plant production, in food processing operations or from the biocides used in general sanitization and disinfections [2], including animal carcasses in some countries. The mechanisms of resistance and adaptation of microorganisms to biocides are unknown, suggesting the need of further investigation of the phenomenon in the actual food systems. The long-term effects of extensive sanitizer use in food processing environments are still on debate, but in relation to the benefits and risks from its use as antibacterial agents in hospitalized humans, [2] S.B. Levy (2002) believes it should be reserved to the most vulnerable patients during recovery, and at
home only. Resistance to quaternary ammonium compounds are associated with the development of multidrug resistance in gram-negative bacteria [283].

Animal production is primarily a business to manage the financial investment and regular expenses with the productive factors, (which are particularly enlarged by feed, medicines, and veterinarian assistance) to obtain the maximum profit and the minimum losses possible. The main objectives of farms are to prevent subclinical and/or clinical disease in animals, to guaranty feed efficiency, (enhancing the animal’s growth in some countries) and to produce safety food, with the quality people want to find and can buy. Growth promotors have been banned in the EU since 2006 [1,121] when the phasing out of the antimicrobial feed additives has been completed and it is not likely that such substances are continued to be used illegally because regular official controls often search for its detection, even at vestigial levels.

With the current available formulations and strengths of the veterinary antimicrobial medicines regularly used, a consistent promotion of animal’s growth with those agents would just represent a decrease in the feed expenses not covered by the veterinary medicines’ expenses. Besides, the additional risk of residues concentrations above MRLs in foodstuff, may be a strong disincentive for such illegal practices.

Nevertheless, this unlikelihood is not a certainty and although it can’t be proved, some antimicrobial consumption profiles suggest that the occasional usage of certain classes of antimicrobials are indeed a legal “growth effect pressure” [292].

The prophylactic use of modern antimicrobial retard formulations with minimal withdrawal periods may increase on crucial moments of the animal’s lifecycles, often related with reproductive stages and movements, particularly prior to slaughter, when maximum investment has been done and losses may be too heavy for the business sustainability [110,309,310]. This procedure should be reverted rationally and without
implicated limitations because the veterinary sector face a serious problem of availability of the veterinary medicines since ever that should not be further aggravated by other “precautionary bans” [117] that favour the illegal use of non-authorised substances. Sales via the internet for instance, are actually under discussion on the ongoing revision of the veterinary medicines Community code, and despite it is already known that it cannot be touched under the concurrence legal provisions, it must be subject to the necessary rules and adequate controls. In the other hand, a “precautionary ban” for the use of antimicrobials, (or some antimicrobials) in food producing animals is not only totally unrealistic as it would increase the production costs, decrease the animal production, increase the food prices to final consumers, eventually with less safety and quality guarantees. In fact, an unknown risk of maintaining antimicrobial use in animals may be less than the risk of reducing that use, suggesting that the relationship between the use of specific antimicrobials in food producing animals, and the resistance selection rates among major foodborne bacteria, should further be studied.

AMR is a health threat, an economic problem and a political issue, despite the economic impact of AMR on the food animal production and on the foodborne diseases aren’t fully investigated, because it is too difficult to assess. The economy affected by the veterinary resistance is not related to the increased health costs and work leaves. It impacts also on agriculture and markets directly affecting countries’ GDP.

Regulators intend to inverse the AMR tendency by monitoring and observing the Community legal acts, establishing specific action plans [299] and monitoring antimicrobial consumption. The information collected on bacterial surveillance and on antimicrobial consumption is very important for risk assessment and for interpreting the follow-up studies of management decisions [114].
The AMR emergence is an emerging issue recently accentuated by the increased occurrence of multi resistant microorganisms, mostly in human beings and in view of a significant statistic relation between the overall amounts of antimicrobial agents consumed and resistance emergence, related in particular to some pharmacological classes [299].

Although the selection of AMR has been mostly studied at concentrations above the MIC, resistant strains are also selected for over a wider concentration range, including those much lower than the MIC.

In the veterinary sector, no data on sales or consumptions trends were available until very recently (2010) [299,300,301] but in order to alleviate the selection pressure on AMR it was assumed the need to reduce the overall use of antimicrobials, by using it in the most judicious way, and by establishing or strengthening effective and integrated surveillance systems to monitor AMR and the antimicrobial consumption. Nevertheless, further studies are needed to scientifically document the statistical hypothesis that by reducing the amount of antimicrobials used, and their selective pressure it will consequently contribute to control the incidence of resistant bacterial strains [10].

When monitoring antimicrobial consumption in animals appropriate indicators, others than resistance data, which are essential, should also be considered within the various epidemiological contexts. It is difficult to correlate antimicrobial resistance among foodborne pathogens with particular types of antimicrobials used on farms and even more difficult to compare year-to-year resistance trends, without correlating it with disease prevalence and with the corresponding antimicrobial shifts.

The veterinary antimicrobial consumption surveillance is still not fully implemented in the EU-MS but several countries have already established national programmes for monitoring their consumption and the in vitro susceptibility of animal pathogens against
antimicrobial agents [300], other than those covered by the current Community resistance surveillance in zoonotic and indicator bacteria. The VetPath programmes, organised by the European animal health study centre (CEESA) is the only ongoing European resistance monitoring programme focused on a broad range of bacterial pathogens causing relevant diseases in livestock and poultry while the ComPath is the first pan-European collection of isolates from the main dogs’ and cats’ pathologies [131,132,164].

Integrated surveillance systems contribute for the implementation of adequate sanitary measures against resistant bacteria and permits also to assess the long-term trends of resistant bacteria evolution. In animals, particular attention is given to pathogens, considered to be under greater selection pressure, but also to the intestinal flora (as the major reservoir of indicator bacteria to detect new resistance traits), and to the zoonotic bacteria which can be transmitted to humans by direct contact or via oral route with contaminated food. However, the surveillance of animal resistant bacteria and the antimicrobial susceptibility testing methodologies, urgently need to implement harmonised methods, in order to provide quantitative data which can be compared and consolidated [163,164]. In veterinary surveillance systems, epidemiologic programmes may be established either to detect the emergence of new resistance mechanisms or to follow the clonal spread of a particular phenotype of resistant bacteria [114] despite the spread dynamic between the farm niches and the ecosystems remain unknown.

International and national campaigns have exhorted the prudent use of the antimicrobial medicines to preserve their effectiveness for serious and life-threatening infections without prejudice of the human and animal health and the EU has been taking several initiatives towards the more efficient European surveillance network.

Quantifying antimicrobial use in food producing animals is a very complex process and although limited, the available data suggest that the animal production may be responsible
for a significant proportion of the total antimicrobial consumption. In the ninety’s it was estimated [16,154] that half of all antimicrobials that were sold, were consumed by animals. This percentage *per se* doesn’t permit to infer whether these amounts were excessive or not, in relation to the human consumption. Presumably, it excludes already the percentage of those antimicrobials used in agriculture, which is not clear because the designation is often used in the literature, ambiguously for veterinary medicines, crops and aquaculture.

Nevertheless, it is essential to monitor annual consumption, to characterize the animal species consumption profiles, and to analyse the consumption trends, on the different epidemiological contexts, to conclude whether or not the veterinary antimicrobials are overused, as usually stated.

It is not only important to know how much antimicrobials are used in animal but which are used and how and when they are used, because the impact of the amounts consumed in animals will depend on its most responsible use and on the preservation of these substances by reducing it whenever possible and as much as possible [161]. The risk of establishing too ambitious quantitative goals for such reduction, often fails to include relevant risk factors related to animal health and may thus have immediate serious public health impacts if not adequately balanced. Intensive but progressive and prudent de-escalation of the veterinary antimicrobial consumption seem to be more feasible, more reliable, and more cautious providing that adequate measures are simultaneously being taken and ensuring the main role of the veterinary public health. On the other hand, when high percentage decreases are imposed for antimicrobial consumption, it may dissuade the misuse or abuse of antimicrobials or it may encourage illegal use as well.

Complex political, economic, and social barriers have limited the quality and the availability of information on the use of antimicrobials in food producing animals. Today,
such data are being provided on a voluntary basis, through the ESVAC project network and the methods used to collect it, to analyse it and to report results are harmonized in the EU [295]. Further work and research will be necessary to provide more information on links between the use of antimicrobial agents in animals and antimicrobial-resistant infections in humans, and *vice-versa*. And this is why the veterinary data have to be comparable with the human data, on antimicrobial consumption. Such comparison should however consider many relevant differences, before drawing any linear and serious conclusions.

Ultimately, the harmonized collection of data obtained in different countries will certainly contribute to improve knowledge and lead to a better and rational use of antimicrobials [9]. In addition, whenever necessary, it will support the amendment and the optimization of ongoing risk assessments and regulatory measures [303] that might have been taken for AMR containment, may improve the terms of the marketing authorisations granted for antimicrobial veterinary medicines and mostly in particular, it should assist the Community legislation on veterinary medicines and on medicated feed, currently under revision at the EU Council level since 2014 and eventually, the water directive to include the antimicrobial monitoring.

Implementation of surveillance programmes on antimicrobial consumption and on resistant bacteria will provide valuable information that must be integrated [1,11] to facilitate the determination and evaluation of the relationship between the use of specific antimicrobials in food producing animals and the resistance selection rates among major foodborne bacteria at slaughter and on farms, and to improve the ability to predict the potential for cross-resistance, by determining and better understanding the mechanisms of resistance. These mechanisms are also key factors to be known and used against AMR development.
Data on veterinary antimicrobial sales and consumption should therefore be robust in order to obtain credible and conclusive results, from reliable sources. The sales collection data is annual, without an automatic system in the majority of the EU-MS. After completing the ESVAC model, data sources are not inspected to detect any possible errors or inaccuracies. Considering that all veterinary antimicrobial agents are POM (prescribed only medicines) under the EU legislation, the veterinary electronic prescription would certainly be the best way out to overcome this issue and to obtain accurate data on veterinary medicines sales and consumption at a time [176]. This methodology would also facilitate the controls on veterinary medicines trade and on food safety related aspects.

Whereas the monitoring purpose is the analysis of routine measurements and observations to detect eventual changes although not eliciting responses, the aim of surveillance is to demonstrate and identify the distribution of the antimicrobial consumption in each country or region, by species, life-cycles and active substances in order to allow timely dissemination of information for integrated action among the different sectors (AMR, residues, quality, zoonosis etc.).

The ESVAC was a milestone project with a high level of harmonization among the European countries for the collection and report of data on sales of veterinary antimicrobial medicines. Currently it has also an ongoing work for collection and report of data on antimicrobial consumption by animal species by using an international technical unit of measurement equivalent to those already used for human medicines (DDD) to be compare animal and human consumptions.

A pilot phase to collect antimicrobial consumption data in pigs was already implemented in few volunteer MS but [18] it was temporarily discontinued requiring further refinement of the project.
The ESVAC model for antimicrobial consumption data collection in pigs, was also tested for the present thesis and the overall conclusion matches the need to review some structural and functional aspects that may pose difficulties to countries to collect and provide national data, if the accuracy of results is not ensured.

Antimicrobial resistance is now a global health issue requiring a global and holistic approach for containment, all over the world. For individual countries it may be also a political concern or a national flag, promoting health systems, trade, tourism, culture etc. The core of the ESVAC project are the main national contact points and data managers nominated by the national competent authorities. It is consequently a EU-MS, EMA and the EC network fitting together the best science, regulatory expertise and veterinary experience on antimicrobial veterinary medicines but without parallel with the WHO structure as an independent and international organization, in charge of the human DDD establishment and review that lead with global health responses, like the OIE which is a mere observer at the ESVAC network.

The lack of harmonization regarding the veterinary medicines supply in the EU-MS hasn’t been a major difficulty for the project to monitor veterinary antimicrobial sales but even tough, the collection of harmonized, and comprehensive data from all countries, into a unique data collection model, took about one year to be completed and endorsed by the NCAs. In relation to the data collection model for antimicrobial consumption in animals by species, increasing difficulties are expectable. In the light of the actual Community legal frame, some provisions may cause per se inaccuracies on results regarding, in particular the medicated premixes and the medicated feed, under the scope of two different legal acts, and ruled by distinct EC General Directions. Additionally, the EMA´s legal competences exclude medicated feed.
A medicated premix is a veterinary medicine covered by the Community veterinary medicines’ code, but after mixed with feed to produce a medicated feed, it is immediately subject to a quite different legal scope, more similar to the animal feed legislation and with significant differences, particularly regarding sales and controls. Medicated feed manufactures have to be authorised feed manufactures or auto-producers as a prerequisite. The quality parameters that are required to produce and to sell medicated feed under prescription, are nowadays a disincentive for the sector. As a consequence, this activity is being progressively discontinued and centralized often outside the boarders, due to the medicated feed free trade rules within the EEA. Official certificates that are issued in the origin to be controlled by the receptor country, (Figure 21), must accompany the consignments and could be used to monitor real consumption of antimicrobial medicated premixes in each country.

Figure 21 – Certificate of medicated feed intra Community trade
Prescription is also mandatory for medicated feed, although it is not necessarily a veterinary prescription in all countries and particularly regarding the medicated feed to be consumed in aquaculture.

Considering that one of the ESVAC objectives is to monitor the antimicrobials sales in Europe, allowing comparisons across the EU-MS, it should be explicit that there may be countries where medicated feed manufactures are inexistent and still, regularly consume medicated feed that has been manufactured in another country, whereas there are countries where medicated premixes sales are higher because it will be consumed as medicated feed in other countries too. Thus, the ESVAC’s sales monitoring may give an overall view of European countries’ consumptions trends but results are not accurate regarding individual countries. Alternatively, the ESVAC’s sales data collection model could be improved by monitoring medicated feed [75] instead of medicated premixes, based on the medicated feed official prescriptions and legal certificates for intra Community trade (Figures 3 and 21). Moreover, antimicrobial sales are the proxy of the antimicrobial consumption per animal species in each country. Data to be collected on antimicrobial consumption by animal species, will probably reveal inconsistencies between sales and consumption profiles and can also be refined. Accuracy is critical for the implementation and development of the ESVAC project, which is politically more complex than the homologous ESAC and structurally too, because it needs to be adapted to several animal species. In addition, antimicrobial consumption surveillance in animals, based on sales (prescriptions, records and receipts), does not necessarily correspond to effective use in animals, because it doesn’t exclude left-overs, incompliances with the label indications or prescriptions, the use of human antimicrobials, and the issue of the multispecies antimicrobial presentations.
In fact, and for a long time, the multi-species presentations of most of the veterinary antimicrobial medicines were considered a barrier to properly monitor the antimicrobial consumption in animals. Nowadays, to overcome such issue, it is considered the possibility to estimate the consumption per species, based on an approximate allocation of the proportion of total sales that are used in each species for which an antimicrobial is indicated by a direct attribution of sales for those products authorised for only one animal species and on attribution of proportions of sales to each major animal species in cases where it is authorised for multiple species. The analysis and report of stratified sales data, using the established DDDvet and DCDvet and an appropriate denominator, is considered to reflect the actual risk of exposure of the different species to antimicrobials and it is already seen as an added value to the European surveillance system for the analysis of the relationship between sales of antimicrobials by animal species and resistance \[18\]. The use of the stratified sales approach to overcome the veterinary antimicrobials´ multi-presentation issue in order to access animal consumption by species reflect a lack of accuracy.

The ESVAC data collection model gives also important information on the most used pharmaceutical forms and on the treated target animal species in the EU/EEA. It confirmed that the injectable preparations are widely used in companion animals and in food producing animals, despite being rarely used in poultry and in other extensive raised species or fish. Oral formulations in general, potentially exert higher selection pressure on resistance due its impact on the animals gut flora and are actually the most sold presentations in the EU. In food producing animals, water as a vehicle, seems to be more effective than feed because dosage may be inferior and also facilitates the intake by diseased animals, usually anorexic and thirsty when hyperthermic, despite the individual appetite to water is also difficult to properly monitor, and the stability and homogeneity
of the mixtures may be affected by the delivery pipe systems. Long action preparations, often available amongst the most modern antimicrobial formulations, are being increasingly used in detriment of the use of older antimicrobial substances with less AMR impact, because are easier to administer and permit to establish shorter withdrawal periods [154]. For Sanyog Jain (2013) [19], the nanodrug delivery system such as liposomes and nanoparticles would be a rational approach to combat multidrug resistance in microorganisms, achieving higher therapeutic efficacy at lower doses, and should therefore be exploited. Nano-formulations to be implemented in the veterinary sector would need to be economically sustainable.

Moreover, the ESVAC model still excludes the topical veterinary antimicrobial medicines which have no major impact in food producing animals, providing that the teat-dips are considered differently. Like the medicated feed, the teat-dips are another presentation, which is exclusive of the veterinary medicines and that are ambiguously covered by two different Community legal acts and are differently evaluated across the EU-MS and at national level only. Most of this products, if not claiming any therapeutic indications may be evaluated as biocidal products in the different MS and are not subject to any records in dairy farms, independently of the qualitative composition. Additionally, regarding the companion animals [176], further attention should be dispensed to topic formulations because despite the apparently small amounts of active substances consumed per package, pets are often treated recurrently with ears, eyes and skin antimicrobial topics, carrying and spreading resistant bacteria in their living environment, almost in permanent and close contact with their owners, all their lives [20].

To assess eventual shifts on total sales or on certain antimicrobial classes’ sales, particularly those reserved for human treatments or for animal second line treatments, and to carefully interpret such trends, complementary information from prescribers should
describe the rational for evident deviations. Those shifts may be due to an increased use of certain antimicrobial agents in pets, to the “cascade/off-label use” in food producing animals or to unpredictable animal diseases and outbreaks, or may result from several other causes related to legal provisions, controls, animal population densities, biosecurity measures, geographical location, production sustainability, epidemiology or even consumer’s preferences on foodstuff of animal origin.

Antimicrobials sales are therefore very important to the antimicrobial surveillance system as the data on consumption by animal species is still ongoing at a very early stage and mostly supported on the sales outcomes, still like a proxy.

Data on sales have been used to estimate the weight of active substance consumed by animals, not taking into account the different doses of the different antimicrobials used to treat animals which is fundamental to better understand the developing resistance mechanisms of each substance or class of substances where the quantity isn’t always [2, 283] the most important factor and resistance mechanisms have a major role.

The technical units of measurement of veterinary antimicrobials, to be universally adopted, (DDDvet and DCDvet)) have been established despite all the difficulties resulting from the multispecies presentations of many of the available veterinary antimicrobial medicines, with several dosage regimens and distinct indications among different animal species. Comparability with data consumption of antimicrobials in humans must be ensured but several sectoral veterinary specificities will have also to be taken into careful consideration when comparing and interpreting both data.

For the different animal species and for the specific pathologies or diseases, distinct antimicrobial agents are often required and there are antimicrobial agents that even can’t be used in some animal species. Even when this is not the case, and depending on the target species, strengths and indications of an antimicrobial medicine also vary
considerably, according to the antimicrobial’s potency, the pharmacokinetic characteristics, the available formulations, the MICs and on the infection to be treated too, reason why the collection, analysis and report of data on sales and consumption of veterinary antimicrobial, are particularly challenging. Currently, the trends in veterinary antimicrobials sales are linked to the annual animal demographics in each MS, based on the Population Correction Unit (PCU) calculated by species, age class and production type. For P. Silley at al., (2012) there are a wide variation between countries in the use of veterinary medicines that cannot be explained by differences in the demographics of animals, requiring a further analysis of the antimicrobial use [131,208].

Quantitative comparison of active substances consumed per biomass treated, (animal and human), allow regulators a general view of usage in both sectors but no guidance for more responsible use and for the most adequate management of the AMR phenomenon.

In a case study performed for the present thesis, it was concluded from the evaluation of the antimicrobial consumption profiles in both humans and swine, within representative populations, that the human sector may be actually consuming more amounts of antibiotics, and more substances belonging to critical classes of antimicrobial like quinolones and cephalosporins, than pigs [291].

Reporting animal consumption of veterinary antimicrobial medicines in DDDvet and DCDvet will however represent a substantial improvement over reporting consumption of active substances in tons, because lower consumptions of critically important antibiotics, for instance, does not necessarily represent less AMR hazard, than higher consumption of less critical classes, in both human and animals. Furthermore, related more potent substances or critical classes of antibiotics when administered to smaller, lighter or young animals may give the false impression of a decrease in total drug consumption although treatment intensity might be unchanged or even increased [228].
The AMR pressure that antimicrobials exert in microorganisms is not proportional to consumption levels but it is important to have harmonized measurement references to analysis the measured consumptions within the same patterns.

Meanwhile, technical units for measurement (DDDvet and DCDvet) have been established to allow comparison between veterinary antimicrobial consumption and human antimicrobial consumption, quantified in DDD. The human DDD gives a rough estimate of medicines consumption, allowing the assessment of trends and the performance of comparisons between population groups, despite being almost always a steady compromise based on the available information. Similar criterion should also be followed by the veterinary sector after many unsuccessful attempts.

Before the ESVAC project some countries had already established their own technical units, adapted to their national surveillance systems like the UDD (Used Daily Dose), the PDD (Prescribed Daily Dose), the TI (Treatment Impact), or the ADD (the animal defined daily dose) which system was considered as the precursor of an international veterinary technical unit). However, any agreement was ever achieved among countries to endorse it, because different means of adjusting usage estimates to the number of animals under treatment, were unsolved inconsistencies regarding antimicrobials consumption report. In the other hand, it is also recognized that any of these units (including DDDvet, DCDvet, or DDD), represent real antimicrobial consumption, although it may allow comparisons between and among countries [186].

In 2005, the EC identified the need of welfare improvements in pig’s productive systems, which have contributed to reduce antimicrobial consumption in this animal species. The ESVAC pilot phase on pig antimicrobial consumption data collection run in few EU-MS, within a minimum number of full cycle farms only, instead a minimum number of animals, making the interpretation of a representative population very difficult to assess
results. Invoking a lack of human and economic resources, and in some cases, the inexistence of the necessary number of farms (five) to be monitored, some countries weren’t able to join the pig pilot phase or candidate themselves to continue with the test. Some MS justified this decision on the basis of the “inexistent legal support” [18], to fully accomplish it but the legal frame for collection of that data had already been indicated by the EC referring to a clear political mandate by the Council to start collecting data on use of antimicrobial agents in veterinary medicine and a sufficient legal basis to request the pharmaceutical industry to provide data on sales of antimicrobial agents to the national authorities (Directive 2001/82/EC and Regulation 726/2004). Only in case of data collection from veterinarians, farmers and/or pharmacies, which are likely sources for collecting data per species, there might be a need for additional legislation [192,296]. Thus, the ESVAC project on consumption data collection per animal species was re-planned for 2016-2020 [18].

For the present thesis, the ESVAC consumption data collection model was tested (2013), following the ESVAC principles for the assignment of the technical units of measurement were however tested to quantify the antimicrobial consumption in pigs, per life-cycle and per active substance but the necessary “population factor”, has been identified as a potential hurdle to calculate those amounts mostly in piglets. To overcome the detected difficulties and to avoid major constraints of the national authorities when called upon to report and publish veterinary results, it is necessary further refinement of the ESCAC methods and further harmonization on the equation to calculate the total annual pig population consumption, by extrapolation of the animal sample consumption, considering that animal populations aren’t statistically stable and may vary considerably over the time. Eventually, only the implementation of the veterinary electronical prescription with
continuous and automatic data collection is believed to address a streamline procedure, totalling all the population consumption data.

Either the European or the national statistical databases may and should be consulted on animal demographics but regarding piglets in particular, these animals are regularly subject to a significant number of commercial transactions before breeding or slaughter, amongst different farms and within a remarkably short period of time. Depending on this movements, animals may be subject to repeated “prophylactic treatments”. Furthermore, piglets are often consumed prior to the standard body weight (BW), established by the ESVAC, particularly in some Mediterranean countries, including Portugal. None of these traits, are reflected at the national or European databases.

The calculation of the national pig population, on a farm by farm basis is only possible with the electronic prescription support whereas data extrapolation from a randomized animal sample to the animal national population, requires the average of piglets per litter to be defined and harmonized. Differences on results may be, in any case, significant and may compromise the ultimate goals of the ESVAC project on harmonization.

Further improvements on ESVAC´s model to collect data consumption should though include criteria on a representative population sample, because farm dimensions vary broadly among countries and regions. Some specific productive factors like birth, morbidity, mortality and repopulation rates, and “all-in/all-out” length and frequencies should be considered when determining a population sample, because consumption is about the number of animals that have consumed the veterinary medicines that were sold, at a certain dosage regimen and all these statistics are well known among the swine production sector and across industrialized countries [292]. On the other hand, despite the facilitation for data collection, the full cycle farms, (tested in the ESVAC pig pilot phase), may not represent or reflect countries pig´s production most common systems.
The ESVAC project on antimicrobials consumption per animal species is crucial for action against AMR but it will take yet some time to be fully implemented. Alternatively, the developments already achieved can be used for another kind of temporary approach, based for instance, on consumption trends. Knowing the annual percentages of different veterinary antimicrobials consumed by pigs, respectively allocated by the different life cycles, it is possible to evaluate a trend and to identify outlier uses, that should be the exception to the pattern and therefore justifiable by animal health issues, like diseases outbreaks. It is important to ensure that medicated premixes will be part of this consumption monitoring, because medicated feed represent a significant (if not the highest) percentage of animal treatments in livestock [75].

The European five-year Action Plan against the “Rising Threats from Antimicrobial Resistance”, proposed to strengthen the surveillance systems on AMR, recognising the Impact of Antimicrobial Resistance in the Human Health Sector and in the Veterinary Sector [140,143]. The ongoing strengthening of the assessment and evaluation of the occurrence of AMR in humans, animals and food in the EU, seek for greater cooperation and coordination on early detection, alert and coordinated response procedures regarding pathogenic antimicrobial resistant bacteria in humans, animals, fish and foodstuffs in order to continuously monitor the extent and growth of AMR.

The balance between the benefits and the risks of the antimicrobials use, or non-use, in food producing animals should also be assessed in the action plans against AMR. Organic animal production would not produce enough food for everybody and the availability of enough protein of animal origin to feed the world population in a near future is starting to be questioned. Moreover in organic production some veterinary medicines, including antibiotics, are allowed [304].
The veterinary authorities provide annual comparable data on the occurrence of AMR in zoonotic agents, or others that may eventually present a public health issue, despite the appreciable lack of harmonization on the resistance detection methods and methodology. AMR monitoring and report are harmonized and covers isolates from *Salmonella* spp., *Campylobacter jejuni* and *Campylobacter coli*, and indicator commensal *Escherichia coli*, and *Enterococcus faecalis* and *Enterococcus faecium* in certain food-producing animal populations and in certain food thereof. Specific requirements are also established for a harmonized monitoring of MRSA and ESBL- or AmpC- or carbapenemase producing bacteria in certain of those isolates.

The veterinary sector has already contributed significantly to further investigations on the AMR epidemiology [291], particularly within the ESVAC network.

The creation of a single European market for the veterinary medicines [52] increasing availability and decreasing the off-label use, and the implementation of the electronic prescription would be the minimum facilitator factors to fully implement surveillance on antimicrobial consumption by animals.

In summary:

Resistance develops everywhere and to all antimicrobial agents that are used both in humans and in animals, emerging, amplifying, evolving and disseminating in the environment, farms, human communities and hospitals.

Antimicrobials prudent use in animals includes a set of practical measures and recommendations to comply with the ethical and economic need to protect animal health, to maintain the efficacy of antimicrobial agents, to prevent or reduce the selection, development and transfer of resistance among animals, from animals to humans or from humans to animals, and to prevent the contamination of food of animal origin [79]. Antimicrobials use in animals may have direct and indirect effects on human health and
vice versa. It may have impact in the emergence of AMR when used in animals but it is essential to consider the complex and concurrent interaction of elements in the environment, of human and animal movements and migrations, of few processing steps within farming activities, and of human behavior on food preparation, meat consumption, and susceptibility to infection. The environment is considered to be one of the most important determinant factors to the human population health [304,305,306,307].

With the current knowledge it is still impossible to determine the contribution of veterinary antimicrobials [114,130,136] to the emergence and incidence of resistant bacteria in human beings.

For the time being, the veterinary antimicrobial consumption data is only a proxy of the sales data, which gives a more realistic view of the European status rather than that of the individual European countries, not allowing accurate comparisons or full epidemiological investigations. Data consumption should be continuously and electronically collected in the best interest of accuracy. The consumption patterns, prevalence or trends when compared with the real amounts of antimicrobials consumed by species, will certainly show significant differences and enable more accurate comparisons and epidemiological investigations which are currently important goals to fight AMR.

Additionally, using the ESVAC model for the data collection on antimicrobial consumption by species and following the principles for the assignment of the veterinary technical units, which requires the quantification of the collected consumption data, there are still some level of harmonization that should be further developed. Particular attention should be given to the definition of the national population and subpopulations factors that may cause considerable discrepancies in countries antimicrobials consumption profiles, influencing the interpretation of results and affecting the adhesion to the ESVAC project. The representability of the animal testing sample should also be revisited.
Whenever “harmonization” is mentioned, it often refers to the best practices that may impact on AMR containment and should be followed. Worst practices are however still recurrent in many parts of the world without boundaries for AMR.

In an integrated surveillance system, antimicrobial consumption monitoring needs to be permanently crosschecked with microbial resistance data, preferably not restricted to zoonotic bacteria which has been frequently reported and studied, but regarding also other bacteria that are becoming increasingly frequent and resistant [9], like *E. coli* [234]. These microorganisms may also configure a serious increase of antibiotic treatments in humans and animals. It’s thus urgent to further investigate any possible or potential AMR epidemiological links among men, animals and environment, to maximize all the actions against AMR, alleviating, whenever possible the greatest pressure on prescribers whose first commitment is to human and animal patients [286,303].

Overall, for the veterinary sector, the ESVAC project has developed and implemented an immense and valuable amount of work [131,208] for the European surveillance on AMR bringing visible benefits for the scientific research and holistic management of the AMR phenomenon. Reinforcing the one health perspective.

Reliable data on animal antimicrobial consumption is paramount to understand antimicrobial resistance (AMR) epidemiology, to manage it and to preserve the antimicrobial substances as a world patrimony, for animal and human protection [163,291]. Complementary, accurate data should be required on resistant bacteria, on veterinary medicines residues [79], on animal diseases and also on veterinary medicines quality parameters as well. The Community regulation on veterinary medicines is very specific and linked with several Community directives on animal health, animal feed, residues controls, forbidden substances in livestock, animal health policies, biocides etc.
with a very distinct scope from the governing Community code relating to the medicines for human use.

Within the global AMR concerns and the one health perspective [292], both legislation on veterinary medicines and on medicated feed are on an ongoing revision and will hopefully establish a new legal frame that may contribute to preserve antimicrobials, ensuring Health. The importance of a European surveillance system like the ESVAC, after adequate regulatory enhancement, reinforces the need of a European single market for the veterinary medicines, where the gradual achievement of free movements of the veterinary medicines is foreseen in the EU legislation since 1981 and establishing for that endpoint that the granting of marketing authorisations in several Member States for the same medicinal product should be made easier [52].

Environmental surveillance systems for AMR should also be considered in a near future, and as an integrated part of the veterinary and human surveillance systems. The veterinary antimicrobial surveillance is not for the sake of the AMR only. It is essential for health, for food, and for the economic growth.

It may be concluded that the development, implementation and continuous improvement of integrated surveillance systems in the veterinary sector are vital to ensure compliance with the enforced regulatory provisions and to guarantee safety and security to all human beings and animals, protecting their health under the most adequate legislative framework. This should be the main next step to provide men and science the best information possible, to continue to fight against resistant bacteria efficiently, integrating as much as possible environment, human and animal data [2], with transparency and accuracy.

Despite the use of antimicrobials in food producing animals may represent a risk to human health, it hasn’t been yet characterized such degree and/or relative impact. The benefits
to animal production haven’t been rigorously investigated neither. On the other hand, any contamination of carcasses with foodborn bacteria, at the slaughterhouse and/or during the preparation and packaging of foodstuff of animal origin, are considered not to pose any serious added risk to consumers, if properly cooked and served.

To our current knowledge, which is nevertheless increasingly higher than before the ESVAC creation, the antimicrobial consumption in humans is heavier than in animals, particularly regarding certain classes usually more connected with rapid resistance mechanisms and more frequent resistance phenomenon. Such comparisons between humans and animals shouldn’t however be used to establish any parallels, as the realities of their use are so different and may be so incomparable. The tendency to decrease use in both sectors, must be in parallel.

Veterinary Medicine has a long experience, protecting both animals and humans and it is actively committed against the AMR threat. This is how the “One Health” perspective should progress, giving also the society, a better chance to know why and how animals are treated with antimicrobial substances, in the EU and particularly in Portugal, nowadays.

The ESVAC project has been a valuable milestone for this purpose and it will permit to draw a roadmap for the veterinary sector, by providing accurate information for the most adequate strategies, based on science and on the features of the antimicrobial consumption in animals.

The EU has aligned in the global project to fight AMR. The FDA also announced the final version of its “Guidance for Industry 213,” (2011), laying out how the livestock industry is expected to phase out the small doses of antibiotics that are used day-to-day on food producing animals either to grow faster, or to grow to market weights using less food. However, it seem that this Guidance just ask the veterinary pharmaceutical industry
to remove from the “label indications” that the antimicrobial substances can be used for growth promotion purposes.

(http://emerald.tufts.edu/med/apua/policy/policy_antibiotic_food_animals.shtml)
CHAPTER VI

CONCLUSION

AMR is a global concern and one of the greatest threats to public health worldwide because in the past two decades, the rate of emergence of AMR has far surpassed the progress in the development of new and effective antimicrobials for therapeutic and lifesaving purposes. On the other hand, in order to meet growing demands for food of animal origin, more intensive and integrated production systems are likely to lead to increased usage of antimicrobial drugs and likely to increase of AMR development and spread. The emergence of antimicrobial resistant strains is dependent on different factors: the antimicrobial substance (dosage, frequency and duration) and the organism involved and whether it carries genes that are resistant to that particular antimicrobial agent. There are also evident links between the use of antimicrobials in agriculture and the occurrence of resistance in foodborne pathogens and commensal bacteria, transmitted through the food chain [286].

The risk of AMR development and spread is closely correlated with increased use of antimicrobial agents, particularly inappropriate use. Such risks are known to decrease in the cases where policy changes control the usage of certain antimicrobial agents by decreasing it too. There are however still many gaps of knowledge on AMR dynamics, epidemiology and mechanisms of development and spread in different agriculture production and agro-ecological systems, in the environment and in humans. The food chain and the environment are extremely important factors in the development and spread of resistant organisms. Resistance genes in both pathogenic and non-pathogenic bacteria can be transmitted from food producing animals to humans via food consumption, or via direct contact with animals or their waste in the environment. Transmission via food has
a potential for widespread dissemination and is quantitatively the most important pathway from farms to consumers. The release of AMR genes into the environment, from a wide range of possible sources, is a critical point for control and a valuable area for continuous monitoring, surveillance and governance.

The national and regulatory policies have to be implemented and/or developed in relation to the use of veterinary antimicrobial medicines, building and strengthening capacities for AMR surveillance and consumption surveillance, monitoring both terrestrial and aquatic animals. The improved awareness on AMR and related food safety threats must progress, providing guidance and support to all the animal production stakeholders on good animal husbandry, including health, biosecurity, management, food safety and hygiene practices and promoting responsible and prudent use of antimicrobial drugs since early days at school [187].

The main objectives of strengthening AMR and antimicrobial consumption surveillance and monitoring are to build country capacities to generate national data on AMR prevalence and trends, and to inform on risk-based management decisions. It also support the formulation of appropriate management policies. Surveillance data from available AMR database that also serves as a surveillance tool and platform for the development of standards for AMR surveillance, provides valuable information on global AMR distribution and trends.

The multi-sectoral and multi-disciplinary nature of AMR entails responsibilities share and global activities coordination to address health risks at the animal-human-ecosystems interfaces fitting the One Health perspective [292].

The Regulatory interventions may provide effective means of minimizing the risks of development and spread of AMR. However, the efforts to policy changes are, often marked by competing or conflicting interests that vary widely around the world [187].
Political and economic factors, organization of the food chain, social conditions and others influence mechanisms for approval and use of antimicrobials in humans and animals, differently and in the different parts of the world.

In the EU these days, regulatory interventions aim to control antimicrobial consumption, establishing the adequate metrics to measure it. Some EU-MS are developing analysis of approaches requiring mandatory reductions in veterinary antimicrobial use or restrictions on certain types of antimicrobial drugs for veterinary use. However restrictions on veterinary consumption of those antimicrobial substances that are critically important for human use are already agreed in principle, by all MS. In addition, measures to control spread of resistant bacteria through infection control programmes and other approaches, improving/assuring quality of veterinary antimicrobial drugs, improving prudent use of veterinary antimicrobial drugs, altering prescribing behavior by veterinary personnel, improving prudent application of antimicrobial drugs should also be carefully considered.

The costs and benefits of preventive measures to improve animal husbandry, health management, food safety, hygiene and biosecurity practices also need to be considered and balanced against the possible negative impacts of antimicrobial’s imprudent use, which is not easy to determine because there are different types of antimicrobials, for different usages, different food systems and distinct regulatory systems around the world.

All these concur for different food producing animals systems and control of transmission of AMR bacteria through the food chain [287].

All these measures must be based on science and on risk analysis principles, requiring capacity building to reliably generate and analyse data on antimicrobial use and on AMR and to inform the development and implementation of risk-based policies and risk management decisions [12]. Legislation must be adapted valorizing Health up the most.
This is how the surveillance of antimicrobial consumption in animals should be developed and is also the main conclusion of this thesis.
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ANNEX 1

Legislação de Medicamentos Veterinários: Breve Sinopse
ANNEX 2

Sales of Antibiotics for Veterinary Use in Portugal Between 2006 and 2009.
ANNEX 3

ANNEX 4

Trends in the consumption of veterinary antimicrobial agents in swine.
ANNEX 5

Quality Programmes for Oral Veterinary Formulations.