Universitat Autònoma de Barcelona

Monitoring of sales of antimicrobials for animal use in the EU/EEA and Switzerland, years 2010 to 2016; a regulatory and statistical analysis

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Abstract

1. Abstract

The problems caused by bacteria resistant to antibiotics have increased during the last years reducing the therapeutic options to treat multi-drug resistant (MDR) infectious diseases in humans and animals.

In order to minimise the onset of antimicrobial resistance (AMR) it is necessary to reduce antimicrobial consumption (AMC). Through a One Health approach, reducing the use of antimicrobials in animals can result in a reduction of AMR in animals and humans.

During the studied period, years 2010 to 2016, antimicrobial consumption in animals has been substantially reduced in many European Union/European Economic Area (EU/EEA) countries and Switzerland. This report analyses data from the mentioned years of the European Medicines Agency (EMA) project, the European Surveillance of Veterinary Antimicrobial Consumption (ESVAC).

A significant decrease in sales of antimicrobials for food-producing animals for 27 countries between the years 2010 to 2016, was found with a mean decrease of 17.1% when expressed as mg/kg of Population Correction Unit (PCU).

Countries that had already had a low AMC in 2010 have continued decreasing AMC by 2016.

In the studied period, the reduction of overall AMC has not resulted into a statistically significant reduction of some of the World Health Organisation (WHO) highly critically important antimicrobials (3rd and 4th generation cephalosporins, quinolones and polymyxins), suggesting that further action is required to reduce the use of those important antimicrobials.

A strong correlation between overall AMC and sales of polymyxins was found, suggesting that the fewer antimicrobials sold, the fewer polymyxins consumed, and vice versa.

High use of oral forms of antimicrobials is correlated to high use of antimicrobials. Countries with high overall sales of antimicrobials have a very strong correlation with the use of premixes, and the contrary. Oral use of antimicrobials might be favoured in countries with high consumption of antimicrobials for economical and practical reasons.

The relationship between the antimicrobials expressed as mg/kg PCU and the % of oral sales for group treatment is not linear but exponential, which seems to indicate that very high sales have to be administered mostly orally (in group treatments) to the animals, as individual treatments will not result in such high sales. It also suggests that reducing the % of oral antimicrobials administered orally could result in an exponential reduction of AMU.

Countries with historical collection of AMC data (before 2007, pre-ESVAC) have lower AMC than those that started to collect AMC data later. But collecting data by animal species (according to a set criteria) does not result in a lower AMC during the studied period. This seems to suggest that collecting sales data on AMC is correlated with an AMC decrease which could be caused by many reasons including the activities of awareness in countries that collect such data and the required involvement of stakeholders, from animal producers to veterinarians.

AMC is linked with temperature in the country, which suggests that those countries with higher environmental temperature might have higher AMC. Countries with high environmental temperature should proactively implement policies to reduce AMC.

Sales data on AMC in animals is a powerful tool to raise awareness and knowledge on the use of antimicrobials, but data at farm level allows for the use of better indicators, and implementation of e.g. benchmarking schemas between farms, and better comparison of results on AMC between countries.

Multifaceted approaches including; setting targets, improved biosecurity, benchmarking, vaccination and avoiding the routine use of antimicrobials seem to have strongly contributed to the reduction of antimicrobial use in animals. In the EU/EEA a new veterinary Regulation (Regulation (EU) 2019/6) should facilitate a further overall decrease of AMC in the EU/EEA during the following years.

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I want to thank Professor Mercedes Campillo, an outstanding biostatistician from the UAB that guided me through the difficult statistical analysis of the data I had compiled and taught me how to use the SPSS software, and accuracy in presenting the data of this report.

I especially want to acknowledge Professor Kari Grave, with whom we started the ESVAC project in 2009. I am fortunate that our professional relationship continues in many projects, mostly related to the use of antimicrobials in animals. Prof Grave is always a source of inspiration and challenge in any activity that leads to the improvement of Public Health.

Also to thank my former manager Dr David Mackay who was in charge at the time of starting the ESVAC project, as well as other colleagues with whom I have been privileged to work with on the project.

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There are other colleagues that I am grateful to have worked with, as part of the EMA scientific secretariat of expert groups at the EMA, or at meetings at e.g. Codex Alimentarius, FDA, OIE, TATFAR or WHO, but that I will not dare to list all of them here.

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Disclaimers

3. Disclaimers

The views expressed in this publication are the personal views of the author and may not be understood or quoted as being made on behalf of or reflecting the position of the EMA or one of its committees or working parties.

Throughout this report, the term 'antimicrobial' is used to include antibiotics and antibacterial agents but excludes antivirals, antiparasitics and biocides (including disinfectants). This definition is consistent with that of the ESVAC report but is noted that is not in line with the new veterinary Regulation (EU) 2019/6 [1] which defines antimicrobials as any substance with a direct action on micro-organisms used for treatment or prevention of infections or infectious diseases, including antibiotics, antivirals, antifungals and anti-protozoal.

The term mg/kg PCU is used in an interchangeable manner with the term mg/PCU (without kg) through this report.

This report includes data from Switzerland, which does not belong to the European Union (EU) or the European Economic Area (EEA), but in order to facilitate the reading of the report, reference is made to "EU" or "EU/EEA" countries in a generic manner, and data will include the data of Switzerland.

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Introduction

5. Introduction

5.1. The problem of antimicrobial resistance (AMR)

In 1969 the Joint Committee on the Use of Antibiotics in Animal Husbandry and Veterinary Medicine, chaired by Professor M Swann (1969) [2] issued one of the first reports to address the risk of AMR from the use of antibiotics in animals. The report concluded that the administration of antibiotics to farm livestock, particularly at sub-therapeutic levels, poses hazards to human and animal health.

The report went on to recommend that only antibiotics which have little or no application as therapeutic agents in man or animals and will not impair the efficacy of a prescribed therapeutic drug or drugs through the development of resistant strains of organisms should be used for growth promotion. This was just 30 years after Dr Fleming [3] published the discovery of penicillin. Twenty-one years later in his Nobel lecture, he warned that it is not difficult to make microbes resistant to penicillin in the laboratory by exposing them to concentrations not sufficient to kill them [4].

AMR is a problem of complex epidemiology with animals considered as potential reservoirs of MDR bacteria, especially of Gram-negative organisms [5]. Although for years there has been opposition from some stakeholders to recognise the possibility of transference of resistance between animals and humans it is now clear that resistant organisms, and its determinants, can be transferred between animals and man, and vice versa [6, 7]. In addition, nearly all antimicrobial classes used in animals are also used in humans [8-10], co-resistance between different antimicrobial classes is of importance for the onset of resistance [11, 12] - making recommendations on prudent use of specific antimicrobials more complex and requiring a One Health approach [13, 14].

It is not possible to estimate to what extent the animal use of antimicrobials has an impact on animal health. The American College of Veterinary Internal Medicine produced a Consensus Statement on Therapeutic Antimicrobial Use in Animals and Antimicrobial Resistance [15] asking amongst other questions, what is the relative contribution of the therapeutic use of antimicrobials in animals to resistance among human pathogens. The answer is far from concrete but shows a consensus between scientist and regulators that use of antimicrobials in animals contributes to the problem of public health of AMR, but that it is not known to which extent.

The second join integrated analysis of the consumption of antimicrobial agents and occurrence of AMR in bacteria from humans and food-producing animals (JIACRA) has produced a detailed statistical analysis of the correlations between the use of antimicrobials and resistance in animals and humans. The results of the report confirm - by the use of ecological studies - the correlations between all those factors and provides a quantified estimate of those correlations, which are depending on the antimicrobial substance of reference and the bacterium [7].

5.2. Governmental activities on AMR

The United Nations (UN), on its Seventy-first session, gave a political declaration from the high - level meeting of the General Assembly on AMR [16]. The UN has rarely debated public health issues. The declaration recognises that resistance of micro-organisms to antimicrobials that were previously effective is mainly due to the inappropriate use of antimicrobials in the public

health, animal, food, agriculture and aquaculture sectors, as well as lack of access to health services, including diagnostics and laboratory capacity.

The UN asks for a "One Health" approach. The declaration indicates that the keys to tackling AMR are, amongst others, the prevention and control of infections in humans and animals, including immunization, monitoring and surveillance of AMR; sanitation, safe and clean water and healthy environments.

The WHO in its Global Report on surveillance on Antimicrobial resistance [17] highlights that the lack of discovery of new antimicrobials is of serious concern. Figure 1 below shows how the discovery of new antimicrobials has drained:

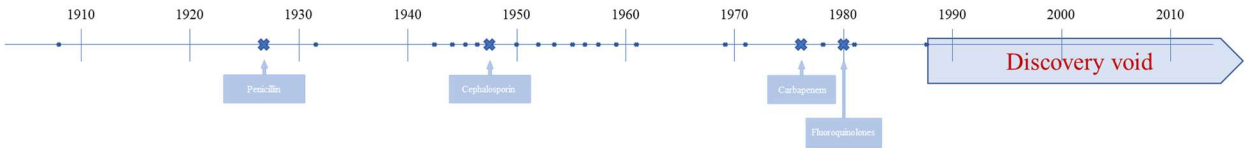


Figure 1. Antimicrobials discovery timeline (adapted from WHO [17]).

The WHO in its foreword of the previously mentioned report indicates that a post-antibiotic era - in which common infections and minor injuries can kill - far from being an apocalyptic fantasy, is instead a genuine possibility for the 21st century. This statement is not to be taken lightly when produced by a trusted institution like the WHO.

The WHO, as well as other institutions and reports [13, 17-27], have identified two main problems related to AMR and public health:

- Very high rates of resistance in bacteria, which can cause common health-care-associated and community-acquired infections (e.g. urinary tract infection, pneumonia).
- Lack of data (e.g. surveillance, standards).

A third one could be added:

- Lack of implementation of risk mitigation measures.

Now that some of the most respected world institutions have identified AMR as one of the most serious risks for human and animal health [28-30], many countries have taken adequate measures to reduce antimicrobial consumption (AMC) and its related AMR [31-33], and as shown in this report some of those have resulted in important reduction of antimicrobial use.

The consequences of AMR in humans are severe; a meta-analysis of relative risk demonstrated significantly increased incidence of delay in effective therapy in extended-spectrum β-lactamases (ESBL) associated bacteraemia [34], the author of the study concluded that in Enterobacteriaceae bacteraemia, ESBL production is associated with increased mortality and delay in effective therapy.

It can be concluded that due to an alarming increase in bacterial resistance, combined with a decline in the development of new antimicrobials, antibiotics are in danger of losing their effectiveness [35]. This would result in a dark future for infectious diseases in humans and animals unless we preserve the available antimicrobials and new antimicrobials are developed [13, 17-27, 36].

In addition to the above, the Food and Agriculture Organization (FAO), World Organisation for Animal Health (OIE), and WHO have established a tripartite Collaboration on AMR [37].

The collaboration addresses the threat of AMR, which rightly considers that it requires a holistic and multi-sectorial (One Health) approach. Recognising that various infectious diseases in animals may be the same or be similar to those used in humans. Resistant bacteria can arise from humans, animals or the environment and may spread between those compartments.

The tripartite indicates: that the WHO, the Food and Agriculture Organization (FAO) and the World Organisation for Animal Health (OIE) speak with one voice and take collective action to minimise the emergence and spread of AMR. With the aim to ensure that antimicrobial agents continue to be effective and useful to cure diseases in humans and animals and to promote the prudent and responsible use of antimicrobial agents.

This joint coordinating activity is very much needed at a moment of many different actions on AMR taking place at the same time by different institutions.

The FAO, OIE and WHO tripartite has produced a manual; "Antimicrobial resistance. A manual for developing national action plans" [38], to assist countries in the initial phase of developing new, or refining existing national action plans.

The manual is intended for national policy-makers, programme managers and partners responsible for strategic planning, development and implementation of national plans and AMR activities in all relevant sectors, this a unique initiative in which the most important international organisations are integrated into one single plan. It also proposes an incremental approach with the intention that countries worldwide, independently of the resources available, can prepare national plans on AMR.

Importantly, the tripartite has produced a library of national action plans[1], where national plans from other countries can be found. This repository of national plans should be handy for those countries that want to develop an action plan on AMR, where they can find action plans from countries in similar situations in relation to, e.g. resistance, resources and other factors of relevance like distribution of medicines, and more specifically in the animal field, animal production or involvement of veterinarians on the prescription and sale of antimicrobials.

The French Plan "Plan national d'alerte sur les antibiotiques 2011-2016" [39] is especially useful. It provides a very detailed, down to earth, plan that could be implemented in countries of the Mediterranean area (and others) without having to reinvent the wheel. As shown in this report, to the credit of the plan, France has achieved a fantastic reduction in AMC since the implementation of the plan (-47.1% reduction between the years 2010 and 2016, see Table 4) [40].

For the animal field, it is especially relevant that the tripartite manual [37] requests that support to farmers and producers is provided in order to adopt good animal husbandry and health management and biosecurity practices to reduce the need for antimicrobial drugs in animal production.

The manual highlights how national plans on AMR should reflect the principles of:

- whole-of-society engagement, including "One Health" approach;
- prevention first;
- ensuring access while avoiding excess;
- sustainability of interventions;

[1] See http://www.who.int/drugresistance/action-plans/library/en/

- incremental targets for implementation.

Those principles are part of the WHO Global action plan on antimicrobial resistance [23] which was endorsed at the 68th World Health Assembly in May 2015 to tackle AMR. The plan has a number of strategic objectives:

- to improve awareness and understanding of AMR;
- to strengthen knowledge through surveillance and research;
- to reduce the incidence of infection;
- to optimize the use of antimicrobial agents; and develop the economic case for sustainable investment that takes account of the needs of all countries, and increase investment in new medicines, diagnostic tools, vaccines and other interventions.
- ensure global access to medicines of good quality.

For many decades the EU has been a leading force in the fight against AMR [20]. Starting with the banning of old-growth promoters by Sweden in 1986, which ended with the total banning of growth promoters in 2006 in the EU [41-45], measures have been taken in Europe to decrease the use of antimicrobials and minimise the problem of AMR [46-48].

Measures to decrease the use of antimicrobials have been traditionally led by Scandinavian countries [41, 49-55], some countries like the Netherlands [56], Germany [57, 58] or France [59] have joined these initiatives with great enthusiasm [60]. The publication of the ESVAC reports has encouraged countries that had identified the problem of high AMC - but had not taken specific measures - to establish plans to decrease AMR. Currently, a plethora of EU countries now have plans to tackle AMR [61-65].

The European Centre for Disease Prevention and Control (ECDC) maintains a web page [66] with all the EU Strategies and action plans on AMR, with direct links to the web page of EU countries as well as a summary of the EC most relevant publications on AMR. Other EU Agencies like the EMA or EFSA (European Food Safety Authority), also keep dedicated pages to the problem of AMR.

The EMA CVMP has had a strategy on AMR for a number of years now, see 5.12.3. [47, 48].

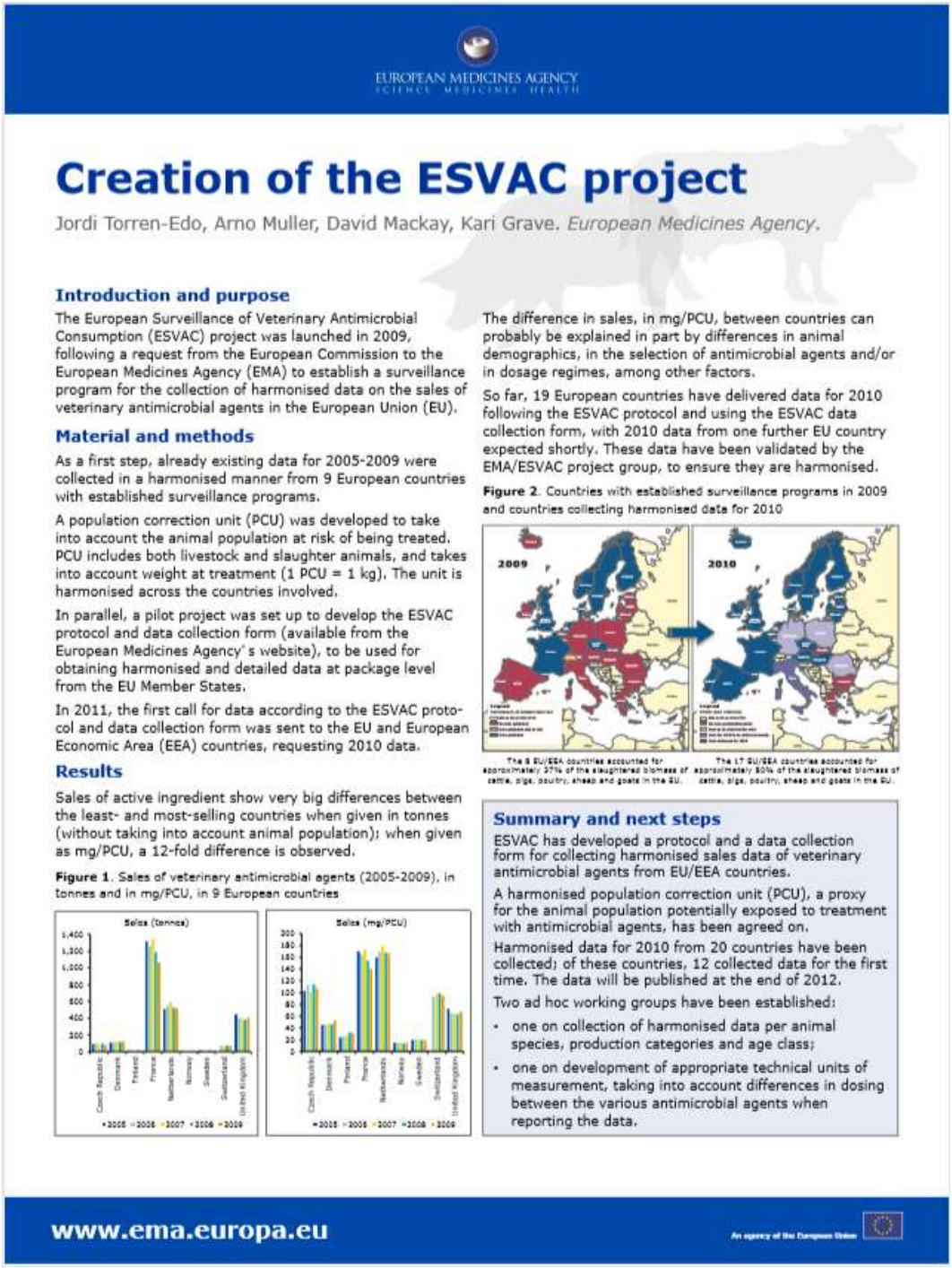
At a worldwide level, the OIE has produced reports on AMC in animals [67-69], the methodology for collecting the sales of antimicrobials are based on the ESVAC template.

5.3. The ESVAC Project

In September 2009, the EMA received the mandate from the European Commission (EC) to develop a harmonised approach for collection and reporting of data on antimicrobial veterinary medicinal products. The request indicated that it should be based on national sales figures, combined with estimations of usage in at least major groups of species (poultry, pigs, veal, other ruminants, pets and fish). It also indicated that it corresponded to the EMA to collect the data from Member States (MSs) and manage the database as well as draft an annual report with the data from MSs.

Almost immediately after, the ESVAC project was established. An annual report was produced with the sales reported by the countries that provide data to the project [61].

The project has grown from collecting data from 9 countries to now reporting data from 30 countries which cover about 99% of the annual animal production in those countries, with only data from Malta missing from the whole EU [61, 70, 71].



EUROPEAN MEDICINES AGENCY

Creation of the ESVAC project

Jordi Torren-Edo, Arno Muller, David Mackay, Kari Grave. *European Medicines Agency.*

Introduction and purpose

The European Surveillance of Veterinary Antimicrobial Consumption (ESVAC) project was launched in 2009, following a request from the European Commission to the European Medicines Agency (EMA) to establish a surveillance program for the collection of harmonised data on the sales of veterinary antimicrobial agents in the European Union (EU).

Material and methods

As a first step, already existing data for 2005-2009 were collected in a harmonised manner from 9 European countries with established surveillance programs.

A population correction unit (PCU) was developed to take into account the animal population at risk of being treated. PCU includes both livestock and slaughter animals, and takes into account weight at treatment (1 PCU = 1 kg). The unit is harmonised across the countries involved.

In parallel, a pilot project was set up to develop the ESVAC protocol and data collection form (available from the European Medicines Agency's website), to be used for obtaining harmonised and detailed data at package level from the EU Member States.

In 2011, the first call for data according to the ESVAC protocol and data collection form was sent to the EU and European Economic Area (EEA) countries, requesting 2010 data.

Results

Sales of active ingredient show very big differences between the least- and most-selling countries when given in tonnes (without taking into account animal population); when given as mg/PCU, a 12-fold difference is observed.

Figure 1. Sales of veterinary antimicrobial agents (2005-2009), in tonnes and in mg/PCU, in 9 European countries

The difference in sales, in mg/PCU, between countries can probably be explained in part by differences in animal demographics, in the selection of antimicrobial agents and/or in dosage regimes, among other factors.

So far, 19 European countries have delivered data for 2010 following the ESVAC protocol and using the ESVAC data collection form, with 2010 data from one further EU country expected shortly. These data have been validated by the EMA/ESVAC project group, to ensure they are harmonised.

Figure 2. Countries with established surveillance programs in 2009 and countries collecting harmonised data for 2010

Summary and next steps

ESVAC has developed a protocol and a data collection form for collecting harmonised sales data of veterinary antimicrobial agents from EU/EEA countries.

A harmonised population correction unit (PCU), a proxy for the animal population potentially exposed to treatment with antimicrobial agents, has been agreed on.

Harmonised data for 2010 from 20 countries have been collected; of these countries, 12 collected data for the first time. The data will be published at the end of 2012.

Two ad hoc working groups have been established:

- one on collection of harmonised data per animal species, production categories and age class;
- one on development of appropriate technical units of measurement, taking into account differences in dosing between the various antimicrobial agents when reporting the data.

www.ema.europa.eu

Figure 2. ESVAC Poster on the ESVAC creation presented at ESCMID in 2012 [72].

As described, e.g. on the ESVAC reports [61-65, 70, 71] and on the JIACRA reports [6, 7] the ESVAC project, coordinated by EMA, collects harmonised data on overall sales of antimicrobial veterinary medicinal products (VMPs) at package level from the EU MSs (except Malta as mentioned) and also from Iceland, Norway and Switzerland.

The sales data are collected from various national sources (wholesalers, marketing authorisation holders, feed mills and pharmacies) and the data are presented by antimicrobial class or sub-class according to the classification system for anatomical therapeutic chemical animals (ATCvet).

Data are uploaded into the ESVAC database, subjected to a standardised validation process and final approval by ESVAC main national contact points.

The reporting countries can at any time upload or re-upload data to the ESVAC database, e.g. for correction purposes.

In 2016, the ESVAC established defined daily doses for animals (DDDvet) and defined course doses for animals (DCDvet) (EMA/224954/201612) [73].

To prepare for the collection of data by animal species, in 2018, the ESVAC published guidance for the collection of harmonised and standardised data from MS on the use of antimicrobials by species [74].

The ESVAC project published in 2016 its 2016-2020 strategy that describes the following steps of the project during the mentioned years [75].

The core of ESVAC sales activity is the ESVAC network of national contacts and alternates, nominated by the national competent authorities in the participating EU and European Economic Area (EEA) countries.

The tasks of the ESVAC national contacts are: to provide sales data to the ESVAC team at the EMA in response to annual data calls; to revise the data in terms of quality and validity, following requests from the ESVAC team; to validate the data applied to calculate the PCU; and to provide comments on the annual ESVAC reports.

In the ESVAC analysis of data, products formulated as tablets are considered as almost exclusively used for companion animals and are reported separately; for this thesis, those tablets have also been excluded. Most of the sales reported to the ESVAC project are mainly for food-producing animals [7, 71].

In line with its strategy [75], the ESVAC project recently started to collect data on stratification of sales data by animal species as part of a pilot project [75, 76].

5.4. Quantifying the use of antimicrobials in animals

In the EU, sales of antimicrobials are collected as the number of active ingredients used per kilogram of animal biomass. The ESVAC activity has successfully established a methodology based on the mg of antimicrobial (nominator) divided by the weight in kg of the animals (estimated biomass) that can be exposed to the antimicrobials.

This has been defined as the Population Correction Unit (PCU) [61]. The PCU is a technical unit that is a proxy for the animal biomass potentially treated with antimicrobial agents. This unit does not take into account the dosing schedule but has the advantage that it is relatively simple to obtain this data without having detailed data on AMC per animal species.

In order to normalise the sales data for the animal population, i.e. to take into account the animal population in which the antimicrobials can be used, a population correction unit (PCU) is used as a proxy for the size of the animal population at risk of being treated. It is essential to highlight that the PCU is a technical unit of measurement, used only to estimate sales corrected by the animal population in the individual countries; where 1 PCU is equivalent to 1 kg of different categories of livestock and slaughtered animals [7, 61, 71].

The data sources used and the methodology for the calculation of PCU is comprehensively described in Appendix 2 to EMA's report Trends in the sales of veterinary antimicrobial agents in nine European countries: 2005–2009 [61].

As a veterinary medicinal products might be marketed for more than one species, the sales data as such do not provide information on sales by animal species, which does not allow for a detailed analysis of the data according to, e.g. different types of production types. The new veterinary Regulation (EU) 2019/6 [1] shall in the future allow for the setting of systems for collection of data at farm level with detailed sales by animal species, not only from a few countries but from the whole EU, however those data might still take some years before being available for analysis and publication (see 5.10.5. for more details).

Quantifying the use of antimicrobials in animals can be done by using different units of measurement [77]. With the aim of providing standardised fixed units of measurement for the reporting of data on consumption by species, the ESVAC project established defined daily dose for animals (DDDvet) and defined course dose for animals (DCDvet) for antimicrobial veterinary medicinal products (VMP) [78], those units take into account differences in dosing of veterinary medicinal products (VMPs).

- The DDDvet is the assumed average dose per kg animal per species per day [79];
- The DCDvet is the assumed average dose per kg animal per species per treatment course [79].

Those measures (indicators) take into account the potency of the antimicrobials, so when comparing the use of different antimicrobials between, for example, countries, factors like the potency of the antimicrobials is considered. In addition, human medicine antimicrobials are reported as Defined Daily Doses, so implementation of such a measure when collection AMC data from animals would allow for a better comparison between the use of those antimicrobials in animals and man.

On the other hand, the DDDvet requires knowledge of use by animal species, i.e. those units cannot be applied unless the data is distributed by animal species, the main reason is that DDDvet is dependent on the dose of administration of antimicrobials, which varies depending on the animal species.

The main aim for establishing DDDvet and DCDvet for antimicrobial VMP was to provide standardised fixed units of measurement for the reporting of data on consumption by species that take into account differences in dosing.

For the use of those units of measurement there is a need to have data at the level of the animal species (i.e. pigs, cattle, and poultry). Until data are collected, or estimated, by animal species it will not be possible to precisely report the use of antimicrobials using the DDDvet or DCDvet as those units are species-specific.

At the European national level, different units of measurement have been defined and used [57, 59, 77, 80-82]. Other areas of the world, like the FDA, did not take into account the animal biomass when reporting on sales of antimicrobial for animal use [83], although their latest activities on the area do take into account the animal biomass [84].

Kasabova et *al.* discuss in a recent publication [85] the Used Daily Dose *vs*. Defined Daily Dose and contrast two different methods to measure antibiotic consumption at the farm level. The publication describes how the Animal Defined Daily Doses, in which a set dose is established for the main routes of administration at a standard weight, is less accurate for measure the exposure of the animals at farm level than the used daily doses, which is based on the amount of antibiotics

used (including the duration of treatment) and the numbers of animals present, however, for collecting the used daily doses, the number of treated animals and the treatment duration should be made available when collecting data at farm level i.e. from each animal treatment.

5.5. Caveats of the collection of sales data

The above-mentioned publication Kasabova et *al.* [85] indicates that placement of sales data in relation to the population at risk requires overall assumptions about the weights of the animals treated and the doses applied, identifying one of the main caveats of the PCU, which is that the animals treated are assigned an estimated weight, which might not be correct and might also affect the mg/kg PCU as the sales of antimicrobials are used in different types of population, and accordingly the mg/kg PCU might change depending on the animal population. As an example, the publication claims that the estimated weight of treatment of chickens is too high (1 kg), whilst chickens are usually treated at an earlier age, something that we can concur.

The use of sales data has been criticised for not allowing sufficiently detailed analysis, Bond *et al.,* 2013 [86] indicate that simple country comparisons, based on total sales figures, entail the risk of serious misinterpretations, especially if expressed in mg per kg, and that the use of more precise model calculations for making such comparisons, taking into account differences in dosages and in farm animal demographics, only slightly reduces this risk. They also list that overall model estimates are strongly influenced by animal demographics and a very inaccurate indication of the true differences in exposure, per animal species. Additionally, they insist that to get an appropriate certainty about the true differences in antimicrobial exposure between countries, it is an absolute necessity to have reliable information about the use per animal species.

It cannot be denied that the collection and analysis of sales data has limitations. As an example, the EC report of a one health country visit to Luxembourg [87] notes that although data suggest that the use of antimicrobials in animals is relatively low, this is likely to under-estimate total use since antimicrobials supplied to farmers by veterinarians based in neighbouring MSs are not included in these data.

The use of sales data, i.e. data provided mostly by Marketing Authorisation Holders or wholesalers, as a direct comparison between countries is as a rough proxy for the real exposure of animals to antimicrobials, but in any case, sales data can provide a very strong understanding of:

- Which antibiotics are used in animals,
- Which are the trends in the use of those antibiotics,
- A rough comparison of the use of antibiotics between countries (not a detailed one),
- An understanding of the different classes of antimicrobials used in different countries,
- An estimation of the total use of antimicrobials to, e.g. compare the use of antimicrobials in animals with the use of antimicrobials in humans.

And overall, they provide a stimulus to countries to initiate policies of antimicrobial reduction.

5.6. Collection of antimicrobial use data per animal species

Data collected at the farm, or close to the point of use (e.g. prescriptions), is the future for the EU and other regions of the world if they want to achieve a detailed and comparable data that allows for a much more thorough understanding, and comparison of the use of antimicrobials in animals.

Whilst collection of sales (or use) data is an essential step in order to promote prudent use of antimicrobials; it is to be acknowledged that the gold standard on AMC by animals is the collection of use data at farm level (opposite to collection of sales data from, e.g. Marketing Authorisation Holders).

A few countries have been collecting said use data in the EU in a consistent manner for some years [49, 56, 82], but currently many other countries are in the process of setting up similar systems [59, 74, 88]. The new veterinary regulation, Regulation (EU) 2019/6 will make such data collection compulsory in the whole EU in a few years [1].

The EMA/ESVAC worked for years on guidance on collecting data by animal species [74], in one of its annexes it details the reasons why data on antimicrobial use should be collected by animal species.

Those benefits are divided according to the entity that can take advantage of those data. The guidance indicates that collecting data by animal species/category to be reported at an EU/EEA level would provide trends in use patterns across the years for defined animal species/categories. However, the guidance warns that direct comparison of antimicrobial use between the MSs should be made with precaution and that available data should be analysed taking into account, e.g. differences in husbandry types (size, technologies, management, etc.).

Data at farm level would allow for a better analysis of the prevalence of resistance and AMC and to analyse the impact of risk management measures taken by MSs allowing for the implementation of effective measures in other parts of the world.

So, as for the sales data, a comparison of the use of antimicrobials between countries is a difficult task that needs to be done with caution. Collection of data by animal species would also allow for the use of more refined units of measurement like the DDDvet or DCDvet. As an example, Postma *et al.,* [80] using DDDs (Defined Daily Doses) and DCDs (Defined Course Doses) identified how products in oral non-feed/water administration category in Germany had a significantly longer treatment duration than in Belgium.

For countries collecting use data other benefits from such collection would be available, such as identifying the off label use of antimicrobials, allowing the comparison of the use of antimicrobials between farms (benchmarking) [77, 89], analysis of different prescribing patterns by veterinarians, the indication of use of antimicrobials, or disease occurrence.

Collineau *et al.*, 2016 [77] describe how data collection on antimicrobial usage should be based on the objective of the data collection. According to the publication, the study objective should define the expected outcome of the data collection.

So, for monitoring usage trends over time, the outcome should be the antimicrobial usage in a given population over a period of time compared to another period of time. For the comparison of the use of antimicrobials between different species or countries, the outcome should be the usage by an individual or given biomass of species of a country in comparison with another species or country over a given period of time. For benchmarking between farms the outcome should be the antimicrobial usage by a given biomass in a veterinary practice/farm in comparison with another veterinary practice/farm over a given period of time, and to study the association between antimicrobial usage and AMR the outcome should be the antimicrobial usage in a population that leads to the selection and spread of AMR over a given period of time [77].

5.6.1. OIE collection of AMC in animals

The OIE has produced reports on AMC in animals [67, 68], the methodology for collecting the sales of antimicrobials are based on the ESVAC template; however, the methodology to estimate the animal biomass that can be exposed to antimicrobials follows a different approach.

The OIE estimates are based on animal production (as well as for ESVAC), but contrary to ESVAC the animal biomass for animals that live less than one year is estimated at the time animals reach the slaughterhouse, this results in higher overall animal biomass.

The possible exposure of the animals to antimicrobials gets then overrepresented as animals are not treated just before they are sent to sacrifice. It can be argued that the ESVAC estimation of the animals biomass at the time of treatment, which in some categories is roughly half the weight of the animals at the time of sending them to the slaughterhouse as done by ESVAC, provides a more pragmatic approach on the estimation of the animal biomass susceptible to be treated with antimicrobials.

However, the OIE approach has also some advantages; the ESVAC estimated time of treatment of the animals is based on figures from Monforts [74, 90, 91] of European animals and are country independent, i.e. not adjusted by the weights of the animals in the country, which means that they are also a rough estimate.

Using the OIE (and FAO) figures on weight at slaughter allows to estimate the weight of the animals in different areas of the world [67], but it does also introduce another factor of uncertainty into the calculations as the estimation of the weight might be subjective.

In addition to those differences, is to note that the OIE data are not provided at package level as for the ESVAC project. In any case, the OIE data collection produces excellent reports that allow for a better understanding of the worldwide use of antimicrobials.

5.7. AMR, a multifactorial problem

Many factors favour AMR; lack of new antimicrobials, indiscriminate use of those (in humans, animals and plants) and growing demand of antimicrobials at all levels.

One of the major problems identified with the use of antimicrobials is that resistance to those is reported immediately after their use as detailed by Lewis, 2013 [92].

Table 1. Date of introduction and resistance first reported for common antibacterials, adapted from Lewis, 2013 [92].

Antibiotic class (an example)	Year of widespread introduction	Year resistance first reported
β-lactams (penicillin)	1938	1945
Aminoglycosides (streptomycin)	1946	1946
Chloramphenicols (chloramphenicol)	1948	1950
Macrolides (erythromycin)	1951	1955
Tetracyclines (chlortetracycline)	1952	1950
Rifamycins (rifampicin)	1958	1962
Glycopeptides (vancomycin)	1958	1960
Quinolones (ciprofloxacin)	1968	1968
Oxazolidinones (linezolid)	2000	2001

The mechanisms of AMR are many, those mechanism are not the subject of this thesis, but include the production of enzymes that deactivate or inhibit the antimicrobials (e.g. β-lactam antibiotics, chloramphenicol, aminoglycosides), an alteration of the membrane permeability of bacteria (e.g. macrolide, vancomycin), efflux pumps that actively pump antibiotics out of the bacteria (e.g. tetracycline, macrolide), alterations to the antimicrobial target sites (e.g. macrolide, sulphonamides, fluoroquinolones, vancomycin) and alterations to metabolic pathways that can compensate for antibiotic effects (e.g. sulphonamides) (adapted from [93]).

An OECD report details that patients infected by antimicrobial-resistant diseases are significantly more likely to develop complications (e.g. +13% limb loss and +71% complications in the central nervous system for infections by methicillin-resistant *S. Aureus*) and to die (e.g. up to 2-3 times higher mortality depending on the micro-organism) (see 5.12.5. and [29]).

5.8. Use and source of antimicrobials

The source of the use of antimicrobials in general (humans, animals and other agriculture uses) might be diverse, but antibiotics are mostly for human or animal use, with an unknown amount used for other agricultural purposes other than in animals (e.g. in crops), as further detailed below.

The second JIACRA (joint report on the integrated analysis of the consumption of antimicrobial agents and occurrence of AMR in bacteria from humans and food-producing animals report) [7] details that 8,927 tonnes are used in the EU, and EEA countries for animals whilst 3,821 tonnes of antibiotics are used in humans in 28 EU/EEA reporting countries.

Figure 3 details the comparison of biomass-corrected consumption of antimicrobials by humans and food-producing animals by country in 28 EU/EEA countries in 2014 (adapted from [7]).

The estimates presented are crude and must be interpreted with caution. Countries with less than 95% data coverage for community consumption by humans were Germany (85%) and the Netherlands (92%). In those countries, the consumption expressed as tonnes, without correction for population or biomass, will be an underestimate.

Austria, Czech Republic, Germany, Iceland and Spain only provided community consumption for human medicine. The average figure represents the population-weighted mean of data from included countries.

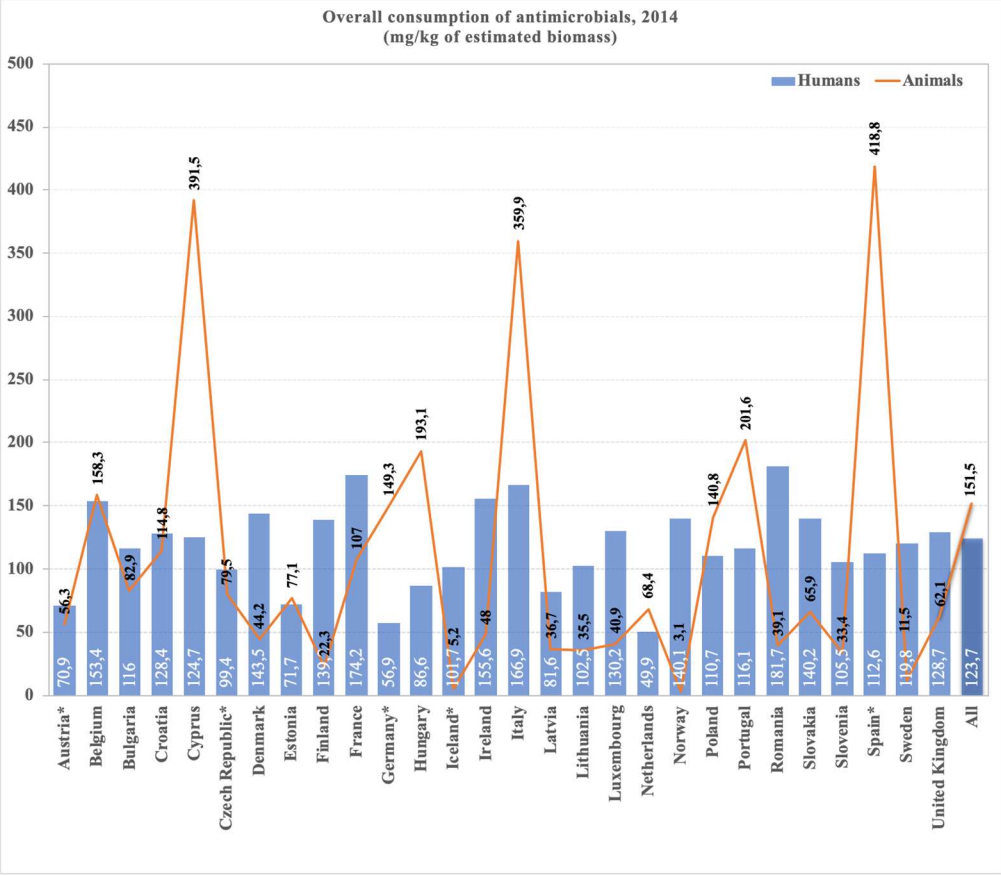


Figure 3. Comparison of biomass-corrected consumption of antimicrobials (milligrams per kilogram estimated biomass) by humans and food-producing animals by country in 28 EU/EEA countries in 2014. Asterisk (*) denotes that only community consumption was provided for human medicine (adapted from [7]).

Data on AMC in humans are collected as DDDs (Defined Daily Doses) by European Surveillance of Antimicrobial Consumption Network [94, 95], but in animals, data are collected by ESVAC as kgs of antimicrobials sold [7] and converted into mg per kg of animal biomass (the PCU).

For the analysis of the human and animal AMC of the JIACRA reports, the antimicrobials consumed by humans, which are collected as DDDs, had to be converted to mg/kg of estimated biomass.

In order to be able to compare DDDs used in humans and animals, there would be a need to collect data in animals as DDDvet [79], this would improve the quality of the comparison, as well as allowing for a much better assessment of the exposure of animals to antimicrobials.

Gut human bacteria are directly exposed to the antimicrobials taken orally during antibiotics treatment. Transmission of resistant bacteria from animals to humans is mostly indirect, via ingesta of resistant bacteria or caused by small amount of residues of antimicrobials in food which might generate resistance in the gut of humans, with the exception of direct transmission of resistant bacteria from companion animals [96] and work-related resistance, like livestock-associated methicillin-resistant *Staphylococcus aureus* [97], which might be transmitted to those working with animals.

Overall the use of antimicrobials is bigger in animals than in humans (8,927 *vs* 3,821 tonnes respectively).

We cannot currently predict the impact on human health of reducing agricultural antibiotic use. Van Bunnik and Woolhouse, 2017 [98] indicate that curtailing the volume of antibiotics consumed by food animals has, as a standalone measure, little impact on the level of resistance in humans and that reducing the rate of transmission of resistance from animals to humans may be more effective than an equivalent reduction in the consumption of antibiotics in food animals.

In addition to the use of antimicrobials in human and animals, there are other uses of antimicrobials that can have an impact on public health. The use of the antimicrobials in plant production is supposedly small and has been described as from 0.2 to 0.4 per cent of total use in agriculture of antimicrobials [99].

According to an OIE publication [100] in 2009 in the United States, 16,465 kg (active ingredient) of antibiotics were applied to orchards, which represented 0.12% of the total antibiotics used in animal agriculture. So, it can be deduced that the use of antimicrobials in crops is small when compared to the use in agriculture; however, is to note that this information is scarce and based on developed parts of the world.

In addition, there is some anecdotal evidence of the use of antimicrobials as biofuels [101-103].

5.9. Impact of the use of antimicrobials in animals on public health

Weese *et al.,* 2015 [15] whilst discussing "What is the Relative Contribution of Therapeutic Use of Antimicrobials in Animals to Resistance among Human Pathogens?", indicate that there is strong evidence that antimicrobial use in animals can promote resistance in some zoonotic pathogens. It also lists that data are far from conclusive and the relative impact of antimicrobial use in various animal species on AMR in human pathogens is inadequately quantified.

A publication from Mather *et al.,* 2013 [104] using whole-genome sequencing analysed a collection of isolates from Scotland from man and animals of *Salmonella* Typhimurium DT104, including a sample of international isolates, studying the phylogenetic associations of the isolates and its AMR genes. The publication suggests that most human infections are caught from other humans (rather

than from livestock) and that in humans, there is a greater diversity of antibiotic resistance than in animals.

Knight *et al.,* 2018 [105] report that the majority of antimicrobial-resistant bacteria are being acquired in the community, suggesting that efforts to reduce overall antimicrobial-resistant bacteria carriage should focus on reducing antibiotic usage and transmission in the community setting. However, the authors do not address the transmission of AMR from animals to humans, so from this study, no conclusion can be drawn on the importance of the use of antimicrobials in animals on public health.

The second JIACRA report [7] confirms the positive association between the integrated analysis of AMC and AMR in both humans and food-producing animals and underlines the need to ensure prudent use to reduce the consumption of antimicrobials in both food-producing animals and humans.

A publication from Dorado-García *et al*., 2016 [106] concludes that epidemiological evidence indicated that drug use history and co-selection of resistance are critical elements for the perpetuation of resistance. Data suggest that recent Dutch policies aimed at reducing the total use of antimicrobials have decreased *E. coli* resistance in the pig and veal calf production sectors while the impact on the dairy cattle and poultry sectors is less clear.

Tang *et al*. [107] in a meta-analysis of 181 studies where the primary outcome assessed was the risk difference in the proportion of antibiotic-resistant bacteria concluded that interventions that restrict antibiotic use in food-producing animals are associated with a reduction in the presence of antibiotic-resistant bacteria in these animals.

Although many data are still missing to complete the puzzle of the impact of the use of antimicrobials in animal health it is clear that there is a need to reduce the use of antimicrobials in animals and consequently reduce the impact of AMR from the use of antimicrobials in animals on public health [20, 21, 47, 48].

5.10. Promoting the prudent use of antimicrobials in veterinary medicine

Recommendations on prudent use of antimicrobials is one of the main risk management tools used within the EU to control the risk of AMR derived from the use of antimicrobials in animals.

Examples of those prudent use recommendations include those provided by the EMA on some classes of highly critically important antimicrobials (CIAs) like fluoroquinolones [108, 109], 3rd and 4th generation cephalosporins [110, 111], colistin (polymyxins) [112-116], macrolides [117, 118], aminoglycosides [119, 120] and pleuromutillins [121, 122].

Those recommendations include e.g. limitations in the use of antimicrobials for prophylaxis, limitations on group treatment, the need to optimise the posology of some old antimicrobials, the need to the revise the rationale for indications for treatment of systemic diseases with substances that have very low oral bioavailability, the need for performing antimicrobial susceptibility testing (AST) before using some antimicrobials or reserving some CIAs for those cases in which other less important antimicrobials for human health are not susceptible.

Some countries have taken rigorous actions like setting targets on the use of antimicrobials [32, 123-134]. Those targets set strict thresholds obligating an active reduction of the antimicrobial use and rely on the capability of those prescribing and administering antimicrobials to implement prudent (or responsible) use recommendations, but also require that AMC is measured properly.

In many cases, the target for the reduction of sales is not only on the overall sales, but the focus is also on reductions on those antimicrobials that are considered critically important by the WHO [135] like cephalosporins, colistin or fluoroquinolones.

In addition to the above-mentioned measures, the marketing authorization of antimicrobials can also be used as a tool for regulating the use of antimicrobials. E.g. by setting the appropriate dose to optimize the treatment of the animals in order to reduce the possible occurrence of AMR.

One of the problems identified in the practice of veterinary medicine and prudent use of antimicrobials is that some Summary of Product Characteristics (SPCs) are outdated with doses that where established many years ago, some of them with vague indications, without proper PK/PD data, or that the pathogens resistance situation might have changed requiring an increase of the dose established [136].

An EMA/CVMP pilot project on dose optimisation of antimicrobials resulted in the Reflection paper on dose optimisation of established veterinary antibiotics in the context of SPC harmonisation [136], the aim of the document was to explore how to optimise the dose of established antimicrobial VMPs by use of PK/PD analysis.

The result of the pilot project on dose optimisation of established veterinary antibiotics was recently published by the Committee for Medicinal Products for Veterinary Use (CVMP), noting that established veterinary antibiotics are not always used at the authorised dose, that doses may need to be reviewed in order to maintain effectiveness and to limit the development of AMR.

The change of the dose of antimicrobial does have repercussions for the whole Marketing Authorisation (MA). Changing the dose of existing antimicrobials might require, amongst other, new studies of target animal safety, withdrawal period studies and environmental risk assessment for old antimicrobials in which the pharmaceutical industry might not be interested in investing which may lead to decreased product availability which could also result in a negative impact on the AMR problem [119, 136].

It is important that veterinarians prescribing antimicrobials have access to all types of antimicrobials, especially those classified by the EMA/AMEG as category D or “Prudent” (see 5.11.2. for further details). If old antimicrobials (like, e.g. penicillins) are not available for the use by veterinarians other substances of highest interest for public health like cephalosporins might be used.

The SPC of a MA might introduce restrictions on the use of certain antimicrobials, e.g. do not use in groups of animals, require an AST of a medicinal product before its use or discourage the use of an antimicrobial, or group of antimicrobials as the first treatment option. The EMA/CVMP has produced a guideline with detailed instructions to be introduced in the information (SPC, package leaflet...) of antimicrobial VMPs [137].

Many countries have introduced both human and veterinary guidelines for the correct use of antibiotics and mechanisms of control of the different families/classes of the antibiotics present on the market today [58, 114, 138, 139], however, those measures are not the subject of this thesis.

The analysis of the EC visits to MSs concludes that benchmarking schemes on antimicrobial use at farm level provide a ready means for veterinarians (and farmers) to compare the quantities of antimicrobials prescribed and used with others in the same farming sectors and for those which deviate significantly from the average which allows to tackle those farms or prescribers where there is an unusually high use of antimicrobials [32].

The Dutch system on controlling antimicrobial use in animals is based on 'traffic light' colours, providing a transparent and straightforward indicator of the relative prescription and use of

veterinary antimicrobials; as a result, it encourages veterinarians and farmers to take actions to be placed in a better category [31, 56, 82, 106, 140].

The well-known Danish 'yellow card' is a system in which herds of pigs have thresholds in use of antibiotics as Animal Daily Dose per 100 animals per day, and when the consumption of antibiotics in a herd exceeds the threshold, the number of annual advisory inspections by a veterinary practitioner increases, and when the consumption of antibiotics in a pig herd exceeds the threshold the farmer gets a warning i.e. a yellow card. The system strongly influences the prescription and use of antimicrobials through establishing thresholds for their maximum use at each stage of the production process [141, 142]. Those schemas are credited with helping to reduce antimicrobial use significantly in the sectors concerned. Some of the farmers and veterinarians working in Denmark have indicated that the thresholds should be set at an appropriate level, with sufficient flexibility in the scheme to avoid potential welfare issues [125].

5.10.1. EC action plan on AMR

In June 2017 the EC adopted the EU One Health Action Plan against AMR [26].

The key objectives of the EC action plan on AMR are built on three main pillars:

- Making the EU a best practice region.

  This is intended to be achieved by having better evidence and awareness of the challenges of AMR, coordination and implementation of EU rules to tackle AMR, prevention and control of AMR, addressing the role of the environment in AMR and improving the availability of antimicrobials.

- Boosting research, development and innovation

  The actions in this area will be; improve knowledge on detection, effective infection control and surveillance. Through developing new therapeutics and alternatives, new preventive vaccines, novel diagnostics, new economic models and incentives. As well as, closing knowledge gaps on AMR in the environment and on how to prevent transmission of AMR.

- Shaping the global agenda

  The objectives in this area of the EU are to provide a stronger EU global presence. Stronger bilateral partnerships. Cooperating with developing countries and developing a global research agenda.

The action plan indicates that the EC will continue to promote animal husbandry, including aquaculture and livestock farming systems, and feeding regimes, which support good animal health and welfare to reduce AMC.

The plan provides the drive for EU MS, Agencies and other stakeholders to continue working on the area of AMR which is a key priority for the EC, the EU governmental body.

5.10.2. The EC fact-finding missions to gather information on the prudent use of antimicrobials in animals

Since 2016 the EC has been visiting some of the EU/EEA countries in order to gather information on the prudent use of antimicrobials in animals. Sometimes those visits have been done under the umbrella of One Health and also compiled information on the use and policies of antimicrobials for human use. Those One Health visits are carried out together with the ECDC.

The countries selected for the visits included those reporting among the highest and lowest sales of antimicrobials to the ESVAC project and several which have reported significant downward trends in recent years.

Until the time of finalising this report the summary of the visits have been published for the following countries: Belgium [143], Bulgaria [144], Cyprus [124], Denmark [125], Finland [126], France [127], Germany [128], Italy [145], Latvia [129], Luxembourg [87], Netherlands [130], Norway [131], Romania [132], Slovenia [133], Spain [123] and Sweden [134].

These reports provide an excellent set of information to understand the use of antimicrobials in those countries in which a visit has taken place, as well as excellent tools for MSs to learn and implement some of the useful measures taken by other MSs.

In 2018 an overview report on measures to tackle AMR through the prudent use of antimicrobials in animals was published [32]. The report summarises the work carried out by the EC Directorate-General for Health and Food Safety regarding AMR in veterinary medicine and reports the efforts from countries to encourage the prudent use of antimicrobials in animals, including a summary of the visits performed until the data of the overview. It highlights examples of potential good practice which may be useful in developing approaches to tackling AMR and the use of antimicrobials in animals in particular. At the time of production of the overview, five of the nine MS visited had AMR strategies in place (note that at the time of drafting this report there were already 16 mission reports available) as listed above. The overview notes that veterinarians play a crucial role in encouraging producers to adopt preventive measures that avoid the need for antimicrobials, while also making informed decisions about the most appropriate antimicrobials to prescribe and identifies how some countries have set overall targets as well as targets for some specific highly CIAs.

It also highlights that the analysis of monitoring AMR and antimicrobial usage shows an association between reductions in AMC and reduced levels of AMR.

The overview tackles the very complex issue of setting targets on reduction of antimicrobial use, indicating that targets for reductions in the use of antimicrobials were set out in several countries, often in conjunction with a clear political commitment to address issues relating to AMR. However, in some countries, the setting of targets was not done claiming limitations in the data available to appropriately set targets for antimicrobial use and to monitor progress as well as the lack of a legal basis for enforcing these targets. Some competent authorities expressed the view that targets should be set based on technical rather than political criteria, in order to avoid problems of animal welfare amongst other reasons.

The overview highlights how voluntary bans or restrictions on the use of certain highly critically important antimicrobials (HPCIAs) in the poultry and pig sectors and avoiding routine treatments of mastitis in dairy cows have been implemented and resulted in the reduction of antimicrobial use in those categories. In addition, the involvement of veterinarians in the development of treatment guidelines not only helped to ensure that they were appropriate but also that they are taken up and accepted by other stakeholders.

Importantly, the overview indicates that data on the sales of antimicrobials for use in animals provides a key measure of overall trends and, more specifically, of the impact of measures to encourage their reduced and more prudent use. It also indicates that the ability to monitor the AMC and AMR changes provides a useful tool for prioritising actions to reduce AMC and AMR and to measure their impact.

On the distribution of antimicrobials, the overview considers as good practices measures that promote closer links between veterinarians and farmers, strengthening the advisory and professional health care role of the veterinarian facilitating long term planning, including planning preventive measures. A greater focus on preventive measures, i.e. better animal health could reduce the need for treatments with antimicrobials.

The overview highlights how considerable efforts have been made by officials, professional and industry organisations, to raise awareness of AMR through guidance and training and that much of the guidance in the prudent use of antimicrobials is readily accessible and can be used by other without having to duplicate the exercise of preparing the guidance.

In line with the EMA and EFSA Joint Scientific Opinion on measures to reduce the need to use antimicrobial agents in animal husbandry in the EU (European Union), and the resulting impacts on food safety (RONAFA) opinion [139], other factors like biosecurity and vaccination are described as primary drivers for a reduced need for the use of antimicrobials and hence resulting in less use of those.

The overview describes how the MSs have declared that in 10 countries, it is mandatory to carry out susceptibility tests to demonstrate that there are no suitable alternatives prior to using CIAs. The implementation of such requirements was reported to have contributed to a substantial reduction in the use of CIAs in several MS visited. For example, in one country, this led to an approximate 99 % reduction in the use of 3rd and 4th generation cephalosporins and a more than 90 % reduction in the use of fluoroquinolones [32].

The overview indicates that following the implementation of measures like benchmarking, setting targets or requiring susceptibility testing for some CIAs, few negative impacts were reported by those who had taken such steps. The major challenges identified concerned changes in management practice, especially when changing from preventive use of antimicrobials to using them to treat individual animals once symptoms appear.

The report concludes on the impact of prudent use measures as follows:

- Substantial reductions in antimicrobial use have been achieved following the adoption of a policy for their prudent use;
- Regulatory intervention is not always required as initiatives are taken by veterinary and industry associations, individual farmers or retailers all contribute to the overall reduction in antimicrobial use;
- Case studies show that adopting an integrated approach incorporating preventive measures and using antimicrobials in accordance with prudent use principles does not adversely affect animal welfare, productivity or profitability in the long term;
- There is scope to better share experience and potential good practices to help others adopt prudent use measures and to identify common critical success factors;
- There is some evidence to suggest that using antimicrobials prudently may lead to reductions in levels of AMR

All the above conclusions make it more relevant to continue analysing the trends on AMC and the different factors that might lead to AMC decrease in animals.

5.10.3. WHO guidelines on the use of medically important antimicrobials in food-producing animals

The 2017 WHO guidelines on the use of medically important antimicrobials in food-producing animals [135] make a series of recommendations in relation to the use of CIAs.

- Recommendation 1: Overall antimicrobial use. The WHO recommends an overall reduction in the use of all classes of medically important antimicrobials in food-producing animals.
- Recommendation 2: Growth promotion use. The WHO recommends complete restriction of the use of all classes of medically important antimicrobials in food-producing animals for growth promotion. Is to note that the WHO recommends complete restriction (the word banning is not used) and only of those medically important antimicrobials.
- Recommendation 3: Prevention use (in the absence of disease). The WHO recommends complete restriction of the use of all classes of medically important antimicrobials in food-producing animals for prevention of infectious diseases that have not yet been clinically diagnosed.
- Recommendation(s) 4: Control and treatment use (in the presence of disease).
    - Recommendation 4a. The WHO suggests that antimicrobials classified as critically important for human medicine should not be used for control of the dissemination of a clinically diagnosed infectious disease identified within a group of food-producing animals.
    - Recommendation 4b. The WHO suggests that antimicrobials classified as highest priority critically important for human medicine should not be used for the treatment of food-producing animals with a clinically diagnosed infectious disease.

Some of the above recommendations are demanding, and it seems unlikely that will be applied in many parts of the world, at least during the next months/years. On the other hand, many of those recommendations should be achievable for some EU/EEA countries during the next years.

5.10.4. RONAFA

On December 2016 the EMA and EFSA adopted a joint opinion on measures to reduce the need to use antimicrobial agents in animal husbandry in the EU, and the resulting impacts on food safety (RONAFA) [139].

The opinion was prepared following a request from the EC [146]. The terms of reference required the EU agencies EMA and EFSA, to review the measures that have been taken by MSs to reduce the use of, and need to use, antimicrobials in food-producing animals, review ‘alternatives’ to the use of antimicrobials, assess the impacts of the measures and alternatives on the occurrence of AMR and to recommend options to reduce antimicrobial use and to promote responsible use.

The RONAFA expert group, was composed by experts from both mentioned agencies and in order to prepare the opinion reviewed information from national antimicrobial use and AMR, ESVAC sales data, EFSA/ECDC AMR reports, scientific publications, literature reviews, surveys and questionnaires as well as data provided by the Food and Veterinary Office (currently EC Directorate-General for health and food safety, DG (SANTE)).

The main recommendation of the opinion is that an integrated, multifaceted approach is taken to reduce the use of antimicrobials in the livestock industry. The opinion notes that no single recommended option will be sufficient to make a lasting impact on the occurrence of AMR in livestock production and its subsequent impact on public health.

Detailed recommendations are made on how to reduce the need for use of antimicrobials (adapted from [147]):

- Develop national strategies and action plans.
- Harmonise integrated systems for monitoring antimicrobial use and AMR in animals, humans and food.
- Establish targets for the reduction of AMU, especially for CIAs.
- Set on-farm health management with professional input.
- Give responsibility to veterinarians for prescribing antimicrobials.
- Increased oversight of preventive and metaphylactic use, especially for groups of animals.
- Provide training and education, raise public awareness about AMR.
- Promote the availability of rapid and reliable diagnostics.
- Improve husbandry and management procedures for disease prevention and eradication; promote the use of vaccination.
- Re-think livestock production systems.
- Develop alternative treatments to antimicrobials.

The RONAFA conclusions are very extensive, a summary of those are included here below:

Successful programmes to reduce antimicrobial use have a multifaceted approach, reflecting the multiplicity of factors that influence antimicrobial use.

Local livestock production systems have to be considered when applying antimicrobial use reduction measures and all relevant stakeholders need to be involved in their implementation.

Some individual measures appear to have had a specific impact in driving a reduction in antimicrobial use in the MSs where they have been applied. These are:

- high-level reduction targets supported in national strategies;
- farm-level measurement of antimicrobial use and benchmarking;
- strengthening controls on group treatments, especially premixes;
- a requirement for antimicrobial susceptibility testing prior to use of high priority CIAs;
- legislative and voluntary industry sector restrictions on the use of high priority CIAs.

The opinion notes that many of the above-mentioned measures have been made mandatory in successful programmes and that supporting measures, such as the provision of treatment guidelines and education, may have been important but have had fewer clear impacts.

At the time of preparation of the RONAFA opinion (drafted during 2015), there was limited evidence about either the positive or negative impact on animal health and welfare of national programmes of reduced antimicrobial use. Since the publication, some reports seem to confirm that antimicrobial use reduction does not have a negative impact on animal welfare [130].

At the time of the drafting of the opinion (2015), there were a few examples where specific measures to reduce antimicrobial use have been associated with a reduction in AMR in bacteria from food-producing animals or foods thereof. For example, cessation of use of 3rd and 4th generation cephalosporins in the pig and poultry sectors were associated with a reduction in the occurrence of extended-spectrum β-lactamase (ESBL)-producing *E. coli* in animals and meat. The

opinion notes that several years of data on AMC are needed before a trend in AMR evolution can be reliably concluded upon.

Marked reductions in AMC achieved in some MSs have had fewer/no impact for certain resistances, e.g. fluoroquinolone resistance in *Campylobacter* spp., and multidrug-resistance in monophasic *Salmonella Typhimurium*, but as noted before, current data seem to indicate that reduction on AMC might result on a reduction of AMR.

Ecological studies have demonstrated correlations between antimicrobial use and resistance in bacteria from food-producing animals [6, 7].

The opinion concludes on the relation between AMC and AMR that overall it is reasonable to assume that a reduction in antimicrobial use will result in a general reduction in AMR in bacteria from food-producing animals and food.

The opinion highlights that the need to use antimicrobials can be reduced dramatically through the application of good farm management and husbandry practices for terrestrial and aquatic animals, and divides those measures in into three main categories:

- to reduce the introduction and spread of micro-organisms between farms (primary prevention),
- to reduce transmission or spread within a farm (secondary prevention), and
- to increase the ability of animals to cope with these pathogens (tertiary prevention).

In relation to alternative production systems the opinion indicates that in the majority of the studies appraised, an association was observed between organic farming and reduced AMR. However, it also notes that due to the limitations in the study design, methodologies for data analysis and biological relevance of the approach, in many of these studies there is a potential for bias in the estimate of the association and effect of organic farming on AMR. Therefore, the RONAFA opinion could not provide conclusive evidence of the impact of organic farming on reducing AMR because of the high level of uncertainty in the appraised studies.

In relation to diagnostic tests, it indicates that some existing diagnostic methodologies are limited by the time taken to obtain results and there are concerns over costs and clinical relevance of the findings. It also notes that the development of modern techniques could enable more rapid and precise diagnosis, allowing better targeted antimicrobial use.

On the alternatives to antimicrobials, the opinion indicates that there are numerous published papers that discuss the potential of compounds and live micro-organisms that may be used as alternatives to antimicrobials in livestock production, but that only a limited number of studies provide robust scientific evidence that conclusively prove that there are successful alternatives. A positive impact on animal health parameters was found for some of the alternatives considered, those substances include: organic acids, probiotics, competitive exclusion, symbiotics, passive immunisation, bacteriophages, immunomodulators, zinc oxide, clay minerals and teat sealants. The opinion already notes that some substances which are used as alternatives to antimicrobials (e.g. zinc oxide) may also increase selection pressure towards AMR.

Finally, the opinion makes a series of overall considerations and recommendations on the conditions for the use of antimicrobials in food-producing animals, which are:

- Antimicrobials remain a key tool for the treatment of infectious diseases in animals.
- In the treatment of livestock, there are three different circumstances for antimicrobial treatment: curative treatment, metaphylaxis and prevention.

- In all cases where administration of an antimicrobial is required, this should be prescribed following appropriate diagnosis by a veterinarian with a good knowledge of the disease epidemiology on the farm and immune status of the livestock.
- Approved treatment guidelines which consider the responsible use of antimicrobials that are CIAs for human health should be followed.
- Animals with clinical signs of a bacterial infection that is impacting their health and welfare in many cases need curative treatment with antimicrobials.
- Metaphylaxis is a strategy frequently used in intensively reared animals and is appropriate when there is potential for high morbidity due to rapidly spreading disease. There should be an aim to refine and reduce the use of metaphylaxis based on identification of underlying risk factors and implementation of measures for their control.
- There should be an aim to phase out preventive use of antimicrobials, except in exceptional circumstances. This should be based on a structured review of such use in each sector/region and development of disease-specific guidance.

The above recommendations on how to reduce the need for use of antimicrobials in animals are a complete toolkit for any country, region or body that wants to reduce antimicrobial use in animals. It is now time to apply those measures, not only at EU/EEA level but worldwide.

In order to facilitate the distribution of the RONAFA message EFSA has produced an interactive web page that provides a high-level summary of the recommendations of the opinion, the web page is available for use by the general public from the following web page; https://www.efsa.europa.eu/en/interactive_pages/Antimicrobial_Resistance.

5.10.5. Legal tools to implement prudent use of antimicrobials in the EU/EEA

5.10.5.1. EU Regulation on VMPs 2019/6

Until recently, the main EC legislation of veterinary medicinal products - Directive 2001/82/EC [148] - included a limited set of rules for the risk assessment of veterinary medicinal products and risk management measures for the control of AMR.

The new veterinary Regulation, Regulation (EU) 2019/6 [1] includes a much more extensive set of tools for assessing the risk of antimicrobials used in animals and tools for controlling such risk.

Recitals 14, 25, 33, 41 to 50 and 93 introduce the reasons and main proposals to control AMR. Those recitals indicate that AMR to human and veterinary medicinal products is a growing health problem in the Union and worldwide, and that it is necessary to mitigate the risk of development of AMR from use of human and veterinary medicinal products.

The legislation is advanced in dealing with the combined use of several antimicrobial active substances which may represent a particular risk with respect to the development of AMR. Even in the EU/EEA, the number of MA for combination products has been reduced importantly during the last years, and the number of products authorised with more than two antimicrobials are scarce, with the exception of intramammaries, for which is not uncommon to contain 2, 3 or 4 antimicrobials.

The ESVAC reports provide details on the number of VMP with more than one antimicrobial, according to the latest ESVAC report [71] of the 9,205 product presentations (tablets excluded) for which sales were reported, 81.5 % contained only one active ingredient, 16.2 % contained two active ingredients, and 2.1 % contained three active ingredients.

In relation to the development of new antimicrobials the Regulation indicates that given the limited innovation in developing new antimicrobials it is essential that the efficacy of existing antimicrobials is maintained for as long as possible and that in order to preserve the efficacy of certain antimicrobials in the treatment of infections in humans, it may be necessary to reserve those antimicrobials for humans only.

In relation to prudent use of antimicrobials the EU Regulation indicates that the supply of veterinary antimicrobials by health professionals should be restricted to the amount required for treatment of the animals under their care and that the identification of risk factors and the development of criteria for the initiation of administration of antimicrobials, as well as the identification of alternative measures, could help in avoiding the unnecessary use of antimicrobial medicinal products, including through metaphylaxis.

Prophylaxis is where the new legislation is stricter indicating that VMPs should not be used for prophylaxis other than in exceptional cases exclusively for the administration to an individual animal. Also for metaphylaxis, indicating that VMPs should only be used when the risk of spread of an infection or of an infectious disease in a group of animals is high and where no appropriate alternatives are available. It also concludes that the restrictions on prophylaxis and metaphylaxis should result in a decrease in its use.

The legislation indicates that there might be a need to prohibit the use of some antimicrobials in animals that are important for the treatment of infections in humans, though they are also necessary for use in veterinary medicine. This is a departure from previous legislation where the specific banning of some antimicrobials for AMR reasons was not part of the legislative tools, or at least not so specifically indicated.

Some of the definitions included in the Regulation are of relevance for the application of the legislation and AMR and are likely to have an impact on the scope and application of the measures that MSs will have to implement. These definitions are:

Antimicrobial resistance: the ability of micro-organisms to survive or to grow in the presence of a concentration of an antimicrobial agent which is usually sufficient to inhibit or kill micro-organisms of the same species.

Antimicrobial: any substance with a direct action on micro-organisms used for treatment or prevention of infections or infectious diseases, including antibiotics, antivirals, antifungals and anti-protozoals.

Antibiotic: any substance with a direct action on bacteria that is used for treatment or prevention of infections or infectious diseases

Metaphylaxis: the administration of a medicinal product to a group of animals after a diagnosis of clinical disease in part of the group has been established, with the aim of treating the clinically sick animals and controlling the spread of the disease to animals in close contact and at risk and which may already be subclinically infected.

Prophylaxis: the administration of a medicinal product to an animal or group of animals before clinical signs of a disease, in order to prevent the occurrence of disease or infection.

The above definitions are the result of many and intensive discussions between all those participating in the drafting of the legislation, especially in what refers to the definition of prophylaxis and methaphylaxis as its definition might have a strong impact on how MSs apply the requirements for limiting the use of antimicrobials in animals.

The legislation provides, e.g. the request of post-authorisation studies for antimicrobials in order to ensure that the benefit-risk balance remains positive given the potential development of AMR, or to refuse a request for a MA when it is for an antimicrobial for use as a performance enhancer in order to promote the growth of treated animals or to increase yields from treated animals.

It also provides an extra period of protection for antimicrobial VMPs for animals when the antimicrobial has not been authorised before as a VMP in animals, the intention of such additional protection period is to support the development of new antimicrobials for use in animals.

Article 57 of the Regulation (EU) 2019/6 [1] includes details on the collection of data on antimicrobial medicinal products used in animals which will have an important impact on some countries that do not have systems to collect data on antimicrobial use at farm level.

The article indicates that MS shall collect relevant and comparable data on the volume of sales and on the use of VMPs used in animals, to enable the direct or indirect evaluation of the use of such products in food-producing animals at farm level. The requirement for comparability and use data is of special relevance as it will require most of the EU MS to set up new systems of data collection at farm level, which will demand substantial investments.

MSs are required to send data on the volume of sales and the use per animal species and types of antimicrobial medicinal products used in animals to the EMA. The EMA is required to cooperate with MS and with other agencies to analyse those data and to publish an annual report. This is something that is already happening for sales data but limited to the willingness of the MSs and only referring to sales data, not to use data (data at farm level).

The EC will have to adopt some pieces of secondary legislation (delegated or implementing acts) detailing the types of antimicrobial VMPs used in animals for which data shall be collected and the quality assurance that MS and the EMA shall put in place to ensure quality and comparability of data. The EC will also have to establish the format for the data to be collected. The EC has mandated the EMA to draft scientific opinions that will be later on be used for the drafting of the above-mentioned legal texts.

The Regulation is certainly ambitious and requires that from January 2024 data be collected at least for the species and categories included in Commission Implementing Decision 2013/652/EU [149] which means collecting data from cattle, pigs, broiler and turkeys. From January 2027, data shall be collected for all food-producing animal species and from January 2030, data shall be collected for other animals which are bred or kept, which in practical terms adds to the previous collection of data for all food-producing species, all companion animals (cats, dogs, gerbils…).

In relation to veterinary prescriptions (Article 105) the Regulation makes compulsory for antimicrobials to be prescription-only indicating that antimicrobial VMPs should only be available with veterinary prescription, and indicates that a veterinary prescription for an antimicrobial VMP for metaphylaxis shall only be issued after a diagnosis of the infectious disease by a veterinarian and that the veterinarian shall be able to provide justification for a veterinary prescription of antimicrobial VMPs, in particular for metaphylaxis and for prophylaxis. It also indicates that antimicrobial VMPs for metaphylaxis or prophylaxis shall be prescribed only for a limited duration to cover the period of the risk. Veterinary prescriptions for antimicrobials will only be valid for five days. All those recommendations are certainly strict and if applied according to the Regulation are likely to result in an immediate decrease of antimicrobial use in animals from the first day of implementation of the legislation.

Article 107 of the Regulation provides a long list of requirements for the use of antimicrobial VMPs in animals. Those include that:

- Antimicrobial VMPs shall not be applied routinely nor used to compensate for poor hygiene, inadequate animal husbandry or lack of care or to compensate for poor farm management.
- Antimicrobial VMPs shall not be used in animals for the purpose of promoting growth nor to increase yield.
- Antimicrobial VMPs shall not be used for prophylaxis other than in exceptional cases, for the administration to an individual animal or a restricted number of animals when the risk of an infection or of an infectious disease is very high and the consequences are likely to be severe. In such cases, the use of antibiotic medicinal products for prophylaxis shall be limited to the administration to an individual animal only.

  The above bullet point means that in practice, prophylactic use of antimicrobials in food-producing species is banned, or nearly banned, especially since in veterinary medicine mostly groups of animals are treated and rarely (e.g. breeders, cesareas) individual animals are treated.
- Antimicrobial VMPs shall be used for metaphylaxis only when the risk of spread of an infection or of an infectious disease in the group of animals is high and where no other appropriate alternatives are available.

The mentioned article 107 also indicates that antimicrobials should not be used outside the terms of the MA (with some exceptions), which depending on the implementation of the regulation could result in the banning (or almost banning) of the off-label use of antimicrobials.

The Regulation also indicates in its Article 119, that antimicrobial VMPs shall not be distributed for promotional purposes as samples or in any other presentation.

Finally, the regulation requires that the potential microbiological risk presented by residues of antimicrobial compounds for the human intestinal flora shall be investigated, something that is already currently done in the risk assessment of antimicrobials but that is now reinforced [14, 150].

5.10.5.2. Community procedures for the authorisation and supervision of medicinal products for human and veterinary use - Regulation (EU) 2019/5

In addition to the new veterinary Regulation (EU) 2019/6, there are other recent Regulations of relevance for the use of antimicrobials in animals.

Regulation (EU) 2019/5 laying down Community procedures for the authorisation and supervision of medicinal products for human and veterinary use [151] includes the requirement for the EMA, the EFSA and the ECDC to publish regularly the JIACRA reports [6, 7], which consist of an analysis of AMC and resistance in the human and animal areas.

The mentioned legislation more specifically indicates in its preamble that since 2015, as the EMA, ECDC and EFSA have published JIACRA Reports, the EMA should continue to contribute to periodic reporting on AMR at least every three years.

It also notes that considering the seriousness of the threat from AMR, it is desirable to increase the reporting frequency within the limits set by feasibility and data reliability.

The JIACRA reports started in 2015 at the request of the EC, until now two reports have been produced [6, 7].

5.10.5.3. EU Regulation on the manufacture, placing on the market and use of medicated feed 2019/4

Regulation (EU) 2019/4 [152] on the manufacture, placing on the market and use of medicated feed, amending Regulation (EC) No 183/2005 repealing Council Directive 90/167/EEC also includes relevant measures intended to reduce AMR, in this case from the use of antimicrobials in medicated feed.

The preamble indicates that it is important to take into consideration the international dimension of the development of AMR.

Interestingly, the Regulation makes reference to Article 118 of Regulation (EU) 2019/6 [1] which provides that operators in third countries are to respect certain conditions relating to AMR for animals and products of animal origin exported from such third countries to the Union. This implies that countries that do not produce animals according to the EU regulations on antimicrobials (e.g. by using growth promoters) may be barred from importing animals and products of animal origin into the EU.

The possibility of the EU banning the entrance of products of animal origin due to use of certain antimicrobials outside of the EU has generated enquiries from countries that export animal products to the EU.

The Regulation also indicates that taking into account the serious public health risk posed by AMR, it is appropriate to limit the use of medicated feed containing antimicrobials for animals. Prophylaxis or use of medicated feed to enhance the performance of animals should not be allowed, except, in certain cases, such as medicated feed containing antiparasitics and immunological veterinary medicinal products, insisting on the restrictions on the use of antimicrobials for prophylactic reasons.

As for methaphylaxis the Regulation indicates that the use of medicated feed containing antimicrobials for metaphylaxis should only be allowed when the risk of spread of an infection or of an infectious disease is high and in accordance with Regulation (EU) 2019/6.

The Regulation also restricts the practice of mixing more than one VMP in the medicated feed and indicates that veterinarian shall not prescribe medicated feed with more than one veterinary medicinal product containing antimicrobials, a practice that has is not uncommon in some countries, in Spain mixing colistin, amoxicillin and zinc oxide in medicated feed for pigs was described as a common practice [153].

Overall, the combination of the three legislations, which include nearly banning the use of antimicrobials for prophylaxis reasons, and the collection of data at farm level for all animals - amongst many other measures - should result on the EU/EEA staying at the forefront on the use of antibiotics worldwide, hopefully all MSs will be able to implement within the set deadlines the requirements of the legislation(s).

5.10.5.4. EMA/CVMP referrals of antimicrobial substances

The use of antimicrobials for the treatment of animals can be partially regulated by the different tools available on the regulations of veterinary medicinal products. Regulation can be established by legislation, e.g. Directive 2001/82/EC on the Community code relating to veterinary medicinal products [148], the new veterinary Regulation (EU) 2019/6 [1], or by guidance produced by other institutions [13, 14, 150].

A referral is a regulatory procedure used to resolve issues such as concerns over the safety or benefit-risk balance of a medicine or a class of medicines.

In the EU, the medicine, or the class of medicines, is 'referred' to the EMA's CVMP so that it can make a recommendation for a harmonised position across the EU, those recommendations might range from the modification of the indications or dose of a substance, or class of substance to the withdraw from the market of an antimicrobial.

The EC following the CVMP recommendations issues a decision to all MS reflecting the measures they need to take to implement the recommendations.

Referrals can be started by the EC, any Member State or by the company that markets or intends to market the medicine.

In the EU, referrals are used to update and harmonise the conditions of use of existing MA to e.g. include prudent use recommendations into the labelling. Referrals are a legal procedure established by Directive 2001/82 as amended, to resolve issues on, e.g. the safety or benefit-risk balance of a veterinary medicinal product (or a class of medicines) [1, 148].

When a referral is launched, by, e.g. a Member State or the EC, the CVMP has to resolve the question addressed. Unfortunately, and due to the lack of data available generated during the marketing authorisation process for old antimicrobial veterinary medicinal products - frequently generic VMPs - those referrals might result in a decrease of the indications and species for which the products are authorised, or even a recommendation to withdraw products from the market. This might result counterproductive from the point of view of AMR as some of those old substances (e.g. some 'old' penicillins) would be the preferred option for the treatment of animals.

The CVMP in its 2015-2020 strategy on antimicrobials [48] indicates that after re-evaluating the benefit-risk for those marketing authorisations of affected products, the consequence of such referrals has been to place restrictions on use, e.g. by removing indications of target species where data do not support use, and strengthening warnings for responsible use and that products which contain combinations of antimicrobial substances, especially if these include CIAs, are of particular concern if their goal is to bypass the need for accurate diagnosis and/or when they are intended for group medication.

In Annex II (Chapter 12.2.) a detailed table of the main EMA/CVMP referrals on systemically (or intramammary) administered antibiotics is provided. The table includes; the year of the end of the referral, the MA (or type of MA), the active substance(s), the target species, the CVMP recommendation and links to relevant web pages from the EU and the EMA.

Those referrals include the so-called "article 35" referrals based on the article of the above-mentioned Directive, some of which might involve a big number of MAs. As mentioned above, some of those referrals have ended with banning products, like the combination of colistin with other substances [154], based on AMR reasons but many others have resulted in changes in the SPC indications or the species for which the products are authorised.

5.11. Categorisations of antimicrobials

When addressing the use of antimicrobials in animals, the importance of those antimicrobials for human and animal health is pertinent in order to establish, e.g. which antimicrobial should be used in order to delay the onset of AMR.

Different lists of CIAs have been produced by different organisations. Those lists provide a ranking of the antimicrobials currently used in medicine for humans and/or animals.

For risk assessors and regulators, the most important criterion seems to be the impact of the use of those antimicrobials on public health, followed by animal health. Other factors could also be taken into account like the impact of the exposure on the environment or the local availability of antimicrobials.

Different institutions like the WHO, OIE, EMA or the FDA have considered the impact of some of those factors and produced lists ranking antimicrobials for use in animals [10, 155-163], those lists might vary depending on the objectives above listed and other factors like the area for which the list is produced, but in most cases the lists are based on the WHO list of CIAs for human medicine, or its criteria, which enhances the importance of such list.

5.11.1. WHO list of Critically Important Antimicrobials

The WHO follows a One Health approach, so its recommendations take into account public and animal health, and the environment.

The first WHO Categorisation list was produced in 2005 by the "WHO working group consultation" with the title "Critically important antibacterial agents for human medicine for risk management strategies of non-human use" [159]. Those lists have been further refined until the latest version which was produced in 2018 and published in 2019 [160].

According to the WHO, the lists of CIAs are intended to be used by authorities, practising physicians and veterinarians, and other stakeholders involved in managing.

Most of the lists that have been produced, including the WHO list, aim to help prioritise risk assessment and risk management.

The WHO list includes as examples of use of the list; prioritizing risk management strategies, including restrictions of use, deciding on off-label use of antimicrobials, prescription status, inclusion criteria in antimicrobial susceptibility monitoring programmes, prioritizing risk profile and hazard analysis activities, development of prudent use and treatment guidelines in humans and animals, and to communicate AMR risks to the public [160, 164].

Importantly the WHO indicates that the lists should not be "considered as the sole source of information to guide a risk management approach".

5.11.1.1. Criteria for the WHO ranking

According to the WHO list [160], the criteria for the ranking of antimicrobials are:

Criterion 1 (C1): The antimicrobial class is the sole, or one of the limited available therapies, to treat serious bacterial infections in people.

The WHO list also includes the rationale for such criteria, highlighting that antimicrobials that are the sole or one of few alternatives for the treatment of serious bacterial infections in humans are important in medicine. It also notes that it is of prime importance that the use antibacterial agents under C1 should be preserved, as loss of efficacy by these substances caused by the emergence of

resistance would have a significant impact on human health, especially for people with life- threatening infections.

The criteria C1 does not consider the likelihood that these pathogens may be transmitted from non-human sources to humans [160].

Criterion 2 (C2): The antimicrobial class is used to treat infections in people caused by either: (1) bacteria that may be transmitted to humans from non-human sources, or (2) bacteria that may acquire resistance genes from non-human sources.

The rationale for such criteria is that antimicrobial agents used to treat diseases caused by bacteria that may be transmitted to humans from non-human sources are considered of higher importance because these infections are most susceptible to risk management strategies related to non-human use of antimicrobials.

5.11.1.2. WHO categories of antimicrobials

According to the 2 above-mentioned criteria, the WHO divides the antimicrobials into 3 categories;

- Critically important: antimicrobial classes which meet the first and second criteria
- Highly important: antimicrobials that meet one of the two criteria.
- Important: antimicrobials that do not meet any of the two criteria.

In addition to the two above criteria the antimicrobials of the Critically Important Category (i.e. those that comply with Criteria 1 and Criteria 2), are prioritised as follows:

- **Prioritization criterion 1 (P1)**: a large number of people in the community or in certain high-risk populations (e.g. patients with serious infections in health care settings), who are affected by *(bacterial)* diseases for which there are very limited antimicrobial choices.
- **Prioritization criterion 2 (P2)**: high frequency of use of the antimicrobial class for any indication in human medicine or in certain high-risk groups (e.g. patients with serious infections in health care settings), since use may favour selection of resistance.
- **Prioritization criterion 3 (P3)**: the antimicrobial class is used to treat infections in people for which there is already extensive evidence of transmission of resistant bacteria (e.g., non-typhoidal *Salmonella* spp. and *Campylobacter* spp.) or resistance genes (high for *E. coli* and *Enterococcus* spp.) from non-human sources.

The WHO also includes a group of substances (classes of antimicrobials) categorized as being highest-priority CIAs. The highest-priority CIAs are those CIAs that meet all three prioritization criteria listed above.

5.11.1.3. WHO lists of antimicrobials by category

As a summary, the classification of classes of antimicrobials includes:

- **Critically important antimicrobials**; aminoglycosides, ansamycins, carbapenems (and other penems), cephalosporins (3rd, 4th and 5th generation), glycopeptides, glycylcyclines, lipopeptides, macrolides and ketolides, monobactams, oxazolidinones, penicillins (antipseudomonal), penicillins (aminopenicillins), penicillins (aminopenicillin with β–lactamase inhibitors), phosphonic acid derivatives, polymyxins, quinolones and drugs used solely to treat tuberculosis or other mycobacterial diseases.
- **Highly important antimicrobials**; amphenicols, cephalosporins (1st and 2nd generation) and cephamycins, lincosamides, penicillins (amidinopenicillins), penicillins (anti-staphylococcal), penicillins (narrow spectrum), pseudomonic acids, riminofenazines, steroid antibacterials, streptogramins, sulphonamides (dihydrofolate reductase inhibitors and combinations), sulfones and tetracyclines.
- **Important antimicrobials**; aminocyclitols, cyclic polypeptides, nitrofuran derivatives, nitroimidazoles and pleuromutilins.

The **highest-priority critically important antimicrobials** include quinolones (in some of the former WHO lists only fluoroquinolones were included in this category), cephalosporins (3rd and higher generation), macrolides (and ketolides), glycopeptides and polymyxins.

The latest version of the list [160], includes a clear flow-chart of the categorisation that helps to understand how the categorisation is applied.

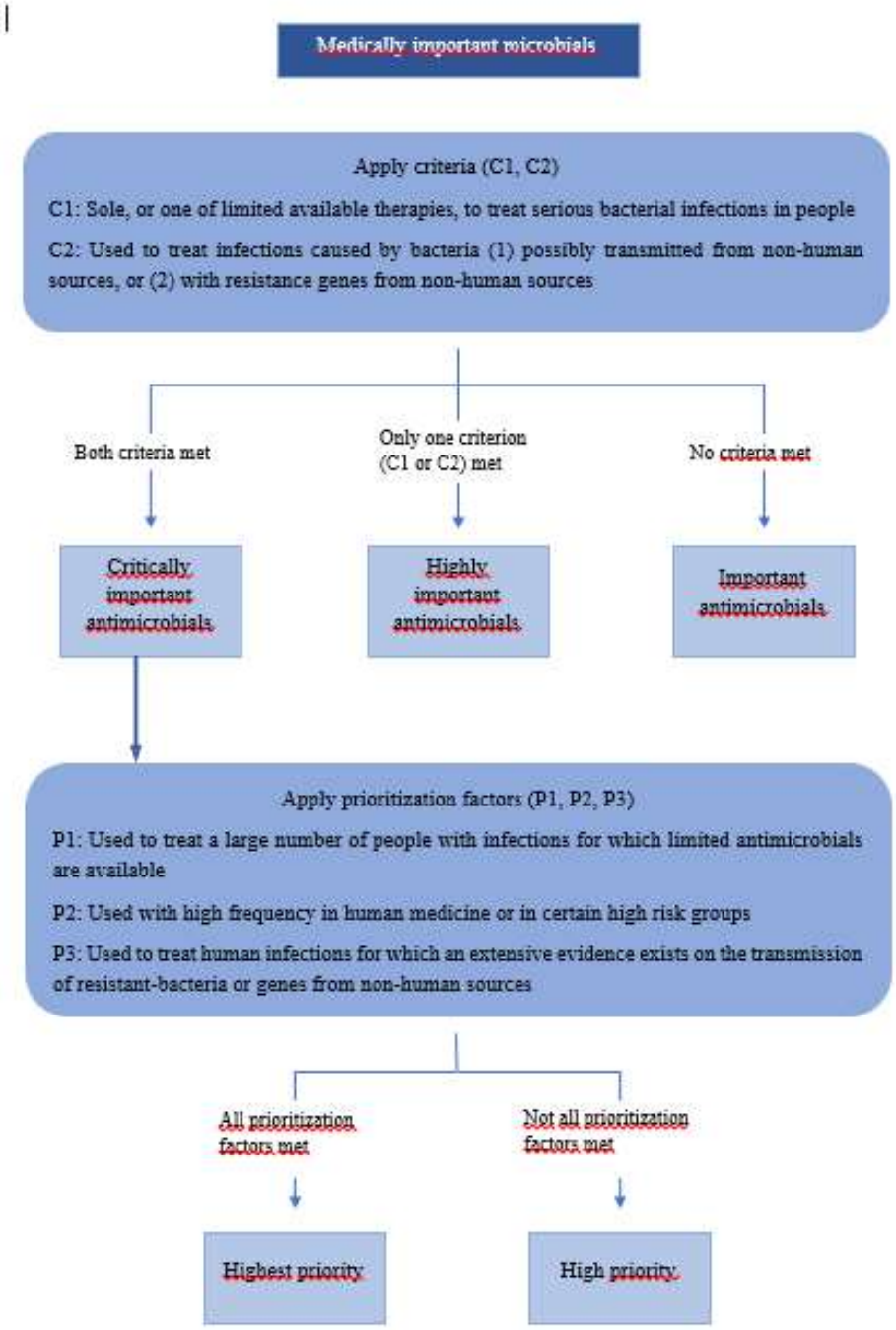


Figure 4. Flow chart of application of criteria and prioritization factors to medically important antimicrobials (adapted from [160]).

5.11.2. The EMA categorisation of antimicrobials

In April 2013, the EC requested advice from the EMA on the impact of the use of antimicrobials in animals on public and animal health and measures to manage the possible risk to humans [165]. The request was divided into four questions:

- Question 1 old antibiotics (colistin and tigecycline)
- Question 2 (ranking of antibiotics)
- Question 3 (new antibiotics)
- Question 4 (risk mitigation options)

The EMA, following the request of the EC, produced in 2014 a categorisation of antimicrobials for use in food-producing animals [9].

The categorisation was part of the answer to a request from the EC on the impact of the use of antibiotics in animals on public and animal health and measures to manage the possible risk to humans (see below under 5.13. for more details on the answer provided).

The advice provided follows a true One Health approach; it was drafted by members of the CVMP, its Antimicrobials Working Party, the JIACRA, the Committee for Medicinal Products for Human Use (CHMP) Infectious Diseases Working party and endorsed by the CVMP and the CHMP. The group was named "Antimicrobial Advice ad hoc Expert Group" (AMEG). The opinions (including the categorisation) were adopted by the CVMP and CHMP in 2014.

The request was later updated and a revised draft adopted by the CVMP and the CHMP and published in January 2019 [10]. The update was to take into account recent EMA/CVMP documents on the risk of resistance and possible impact on human and animal health of the use in animals in the EU of colistin [114], aminoglycosides [119, 120]) and aminopenicillins [166].

One of the main intentions of the ranking was to take into account the use of veterinary medicinal antimicrobials in the EU and to adapt the recommendations to the specific conditions of the region.

5.11.2.1. Criteria for the EMA ranking

For the initial AMEG categorisation [9], two main factors were taken into account; their need in human medicine and the risk for spread of resistance from animals to humans.

To take into account the two above criteria those were addressed as follows:

The hazard of zoonotic relevance (e.g. *Campylobacter* spp., *Salmonella* spp.), probability of resistance transfer (e.g. low or high), use in veterinary medicine (indicating if the substance is approved for use in the EU or not and if authorised if for group treatment and information from MSs MAs.

For each antimicrobial class, it was considered which the bacterial targets are in human medicine in the EU (for which availability of class/substance is critically important due to few alternatives).

For the classification of antimicrobial classes according to their probability of transfer of resistance genes and resistant bacteria, the following parameters were considered:

- Vertical transmission of resistance genes.
- Mobile genetic element-mediated transfer of resistance.
- Co-selection of resistance.
- Potential for transmission of resistance through zoonotic and commensal food-borne bacteria.
- Evidence of similarity of resistance (genes, mobile genetic elements and resistant bacteria).

In addition to the above elements, other factors were considered:

- Apply over the whole of the EU independently of the animal health situation.

- And of the availability of antimicrobial products for animals in the individual MS.

When revising the AMEG categorisation [10], the group took into account not only the CIA antimicrobials but also other antimicrobials. When considering the aminoglycosides and aminopenicillins it was deemed that the addition of an intermediate category was required, especially to improve the utility of the categorisation as a risk management tool by avoiding the counterproductive outcome of too many antimicrobials being placed in a single high-risk category.

In addition, the AMEG group gave further thought to the criteria on the likelihood of transfer of resistance considering if the scoring of the factors taken into consideration was possible and to specific mechanisms of resistance/genes that might have particularly important consequences for human health. In addition to the already existing criteria (the importance of the antimicrobial class in human medicine and the probability of AMR transfer), other criteria were considered. Those criteria were: antimicrobial class (e.g., chemical properties, pharmacological properties…), conditions of use (e.g., animal species; indications, dose and duration, route of administration…), criteria relating to prevalence of resistance (i.e., pathogens, commensals, zoonoses, frequency of resistance, transfer of resistance or mutations), criteria relating to environmental aspects (e.g., degradability of antimicrobials in animals and animal waste…).

Some additional criteria were selected for more detailed consideration: route of administration and indications for veterinary use and availability of alternative antimicrobials of lesser risk.

Considering that antimicrobials in each class are available in a number of different pharmaceutical forms the AMEG decided not to include the route of administration as an additional criterion for the categorisation.

The group noted that nevertheless when factoring AMR risk into prescribing decisions, the aim should be to use the above-list together with the AMEG categorisation to select both the formulation/route of administration and class that will have the least impact on the selection of AMR.

A detailed description of the AMEG considerations on the route of administration are given in the results section under the discussions of the pharmaceutical forms (see section 9.3.).

The AMEG updated criteria was agreed as follows:

- If the (sub)class or group is authorised for use as a veterinary medicine.
- The importance of the (sub)class or group to human medicine according to the WHO ranking and taking into account the EU situation.
- The likelihood and possible consequences of AMR transfer from animals to humans. In the new categorisation individual mechanisms of resistance were considered more specifically for e.g. those genes associated with mobile multi-resistance.
- The availability of alternative antimicrobial (sub)classes in veterinary medicine with lower AMR risk to animal and public health.

5.11.2.2. Categories of antimicrobials (EMA)

The EMA/AMEG categorisation was initially divided into 3 categories, those categories were later revised:

- Category 1: Antimicrobials used in veterinary medicine where the risk for public health is currently estimated as low or limited.

- Category 2: Antimicrobials used in veterinary medicine where the risk for public health is currently estimated as higher.
- Category 3: Antimicrobials currently not approved for use in veterinary medicine.

Category 1 included substances which were considered as the first choice in treatment guidelines and for which no specific associated hazards were identified to which people could be exposed from use in animals in the EU.

Category 2 included substances that should be reserved for the treatment of clinical conditions which had responded poorly or are expected to respond poorly, to other antimicrobials.

Category 3 included antimicrobials currently not approved for use in veterinary medicine (but used for human medicine in the EU), the advice indicated that the extent of use of these classes would be low in the EU due to their legal status according to which these substances may only be used by way of exception and only in companion animals (non-food-producing species) as maximum residue limits (MRLs) have not been established to allow their use in food-producing animals.

Once the categorisation was revised in 2019, a different classification was proposed with four different categories (A to D). In addition, key action words were assigned to each category. The main reason for adding a fourth antimicrobial category was the need to further separate between the use of certain classes of antimicrobials, as it was considered that an intermediate category would improve the utility of the categorisation as a risk management tool by avoiding the counterproductive outcome of too many antimicrobials being placed in the higher risk category.

For aminoglycosides and amoxicillin-clavulanate combinations it was considered whether they should be included in category B (restrict, see below), but the AMEG decided that they presented a lower risk to human health compared to quinolones and 3rd and 4th generation cephalosporins, and added those substances to category C, resulting in the following categorisation:

- **Category A (Avoid)** includes antimicrobial classes not currently authorised in veterinary medicine in the EU.

  In the absence of established MRLs for foodstuff of animal origin, use of these classes of antimicrobials in food-producing animals is prohibited and they may only be administered to individual companion animals exceptionally, in compliance with the prescribing "cascade".

- **Category B (Restrict)** includes the substances listed as highest priority CIAs (HPCIAs) by the WHO with the exception of macrolides and those classes included in Category A.

  This category includes quinolones, 3rd and 4th generation cephalosporins and polymyxins.

  The AMEG opinion (as endorsed by the CVMP and CHMP), considers that for these antimicrobials, the risk to public health resulting from veterinary use needs to be mitigated by specific restrictions. Those restrictions include that the antimicrobials in this category should only be used for the treatment of clinical conditions when there are no alternative antimicrobials in a lower category that could be effective, and that use of those antimicrobials should be based on the results of antimicrobial susceptibility testing, whenever possible.

- **Category C (Caution)** was added as an intermediate category.

  The list diverges from the WHO categorisation, especially by adding to this category the macrolides (instead of in category B), which are considered HPCIA on the WHO list.

  The AMEG indicated that for those substances proposed for inclusion in this category, there are in general alternatives in human medicine in the EU, but there are few alternatives in veterinary medicine for certain indications.

In addition, antimicrobial classes that may select for resistance to a substance in Category A (Avoid) through specific multi-resistance were also placed in this category.

Antimicrobials in category C (Caution) should only be used when there is no substance in Category D that would be effective.

The addition of macrolides under this category - since in general there are alternatives in human medicine in the EU - is why the macrolides have not been analysed with the same scrutiny as 3rd and 4th generation cephalosporins, quinolones or polymyxins in this report.

- **Category D (Prudence)** is the lowest risk category.

  The AMEG also addresses that while the risk to public health associated with the use in veterinary medicine of substances included in this category is considered low, a number of the substances in this category are listed as WHO CIAs (aminopenicillins, natural penicillins and isoxazolylpenicillin).

  The AMEG group acknowledges that these antimicrobials are not devoid of negative impact on resistance development and spread, in particular through co-selection, and indicates that while there are no specific recommendations to avoid use of Category D substances, there is a general recommendation that prudent use principles should be adhered to in everyday practice to keep the risk from use of these classes as low as possible.

  It also indicates that unnecessary use and unnecessarily long treatment periods should be avoided, and group treatment should be restricted to situations where individual treatment is not feasible.

The AMEG categorisation is now to be revised to consider the comments received and produce a final categorisation. A table with a summary of the categorisation is included as one of the Annexes (12.4.).

5.11.3. OIE list of antimicrobials of veterinary importance

In May 2007 the OIE (World Organisation for Animal Health) adopted a list of antimicrobials of veterinary importance categorising the antimicrobials for animal use [161, 162]. The list was further updated and adopted on May 2013, May 2015 and May 2018 by the World Assembly of OIE Delegates.

The list is partially the result of a questionnaire that was sent to the OIE MS and other institutions.

The difficulty of preparing such a list is shown by the OIE's considerations that the list as provided by the MSs had to be revised as initially it included substances banned in some countries, substances not considered critical, and the use of antimicrobials as growth promoters.

The main difference between the WHO and the OIE list is that the OIE list aims to establish the degree of importance for animal use of classes of veterinary antimicrobials. The OIE also notes that one significant difference between the use of antimicrobial agents in humans and animals is the many different species that have to be treated in veterinary medicine.

5.11.3.1. Criteria for the OIE ranking

The first criterion for the OIE list was that a majority (more than 50%) of the OIE Member Countries identified the importance of the antimicrobial class in their response to a questionnaire sent by the OIE.

The second criteria refer to the treatment of serious animal disease and the availability of alternative antimicrobials. The criteria was met when compounds within the class were identified as essential against specific infections, and there was a lack of sufficient therapeutic alternatives [162].

5.11.3.2. OIE categories of antimicrobials

In line with the WHO list, the OIE list is divided between, Veterinary Critically Important Antimicrobials (VCIA), Veterinary Highly Important Antimicrobials (VHIA) and Veterinary Important Antimicrobials. (VIA).

Taking into account the two above-listed criteria, the following categories were established:

- Veterinary Critically Important Antimicrobials: are those that meet both criteria 1 and 2
- Veterinary Highly Important Antimicrobials: are those that meet criteria 1 or 2
- Veterinary Important Antimicrobials: are those that meet neither criteria 1 or 2

5.11.3.3. OIE lists of antimicrobials by category

The three categories of antimicrobials include the following classes of antimicrobials:

- VCIA: aminoglycosides, amphenicols, cephalosporins (3^{rd} and 4^{th} generation), macrolides, penicillins, phenicols, quinolones second-generation (fluoroquinolones), sulfonamides (plus diaminopyrimidines) and tetracyclines.
- VHIA: ansamycin/rifamycins, cephalosporins 1^{st} and 2^{nd} generation, ionophores, lincosamides, phosphonic acid, pleuromutilins, polypeptides, quinolones (1^{st} generation).
- VIA: arsenical, aminocoumarin, bicyclomycin, fusidic acid, orthosomycins, quinoxalines, streptogramins and thiostrepton.

The OIE list [162], also includes specific recommendations for some classes of antimicrobials that are considered to be critically important both for human and animal health; i.e. for fluoroquinolones, 3^{rd} and 4^{th} generation cephalosporins and colistin. Those recommendations are as follows:

- Not to be used as preventive treatment applied by feed or water in the absence of clinical signs in the animal(s) to be treated;
- Not to be used as a first-line treatment unless justified, when used as a second-line treatment, it should ideally be based on the results of bacteriological tests; and
- Extra-label/off label use should be limited and reserved for instances where no alternatives are available.
- Urgently prohibit the use of the above-mentioned classes (or substances) of antimicrobials as growth promotors.

Finally, the OIE list indicates that the classes of antimicrobials in the WHO category of HPCIA should be the highest priorities for countries in phasing out the use of antimicrobial agents as growth promotors.

5.11.4. FDA list of medically important antimicrobials

In 2003, the FDA published the Guidance for Industry, "Evaluating the Safety of Antimicrobial New Animal Drugs with Regard to their Microbiological Effects on Bacteria of Human Health

Concern", for the evaluation of antimicrobial substances for food-producing species #152 [163]. This list was one of the first attempts by a Regulatory Agency to provide a categorisation of antimicrobials.

Appendix A of the mentioned guidance provides the categorisation of antimicrobials according to their importance for antimicrobial use. Interestingly, the guidance from 2003 indicates that the classification is preliminary, the guidance is currently under revision.

5.11.4.1. Criteria for the ranking

The Appendix of the criteria for the ranking defines the criteria as follows:

1. Antimicrobial drugs used to treat enteric pathogens that cause food-borne disease

The Infectious Disease Society of America guidelines on the treatment of diarrhoea and other sources such as the Sanford Guide provides the drugs typically used in the treatment of foodborne diseases.

2. Sole therapy or one of few alternatives to treat serious human disease or drug is essential component among many antimicrobials in the treatment of human disease.

A. Includes antimicrobials like vancomycin and linezolid for MRSA infections. Although they are not the "sole" therapy, they are one of only a few alternatives.

B. This would also include a drug like polymyxins where it is one of few alternatives for MDR *Pseudomonas aeruginosa* infections.

C. Rifampicin is not only a drug used to treat TB, but also it is an essential part of the treatment regimen as the cure rate is lower without it.

D. Serious diseases are defined as those with high morbidity or mortality without proper treatment regardless of the relationship of animal transmission to humans. For example, rifampicin is an essential drug to treat disease caused by *Mycobacterium tuberculosis* (high morbidity and mortality if untreated) even though this is a human pathogen. Gonorrhoea occurs only in humans and is not lethal but can result in sterility if left untreated (high morbidity).

3. Antimicrobials used to treat enteric pathogens in non-food-borne disease enteric pathogens may cause disease other than food-borne illness.

For instance, *E. coli*, which causes food-borne disease, is also capable of causing diseases as diverse as urinary tract infections and neonatal meningitis.

4. No cross-resistance within drug class and absence of linked resistance with other drug classes

A. Absence of resistance linked to other antimicrobials makes antimicrobials more valuable. An example is quinolone resistance in pneumococci, which currently does not appear linked to penicillin resistance. On the other hand, penicillin resistance appears to be linked to macrolide, tetracycline, and trimethoprim-sulfamethoxazole resistance in pneumococci.

B. Cross-resistance within antimicrobial classes and absence of linked resistance may change over time and will need to be updated periodically.

C. In this context, "cross-resistance" refers to the transmission of resistant determinants between bacterial species or genera and does not refer to the transmission of resistant organisms between animals and humans. This is addressed in the release assessment part of the guidance.

5. Difficulty in transmitting resistance elements within or across genera and species of organisms

A. Antimicrobials to which organisms have chromosomal resistance would be more valuable compared to those antimicrobials whose resistance mechanisms are present on plasmids and transposons.

B. This does not refer to “ease of transmissibility” from animals to humans of the resistant pathogen as this is addressed elsewhere in the guidance in the release assessment."

5.11.4.2. Categories of antimicrobials

The annex of the FDA guidance classifies the antimicrobials as follows:

- Critically Important: Antimicrobial drugs which meet both criteria 1 and 2 below.
- Highly Important: Antimicrobial drugs which meet either criteria 1 or 2 below.
- Important: Antimicrobial drugs which meet either criterion 3 and/or 4 and/or 5.

5.11.4.3. Lists of antimicrobials by category

The following classes of antimicrobials are classified as Critically Important by the FDA:

- 3rd generation cephalosporins, fluoroquinolones, macrolides, trimethoprim/sufameth,

The following classes of antimicrobials are classified as Highly Important:

- Natural penicillins, penase resistant penicillins, antipseudomonal penicillins, aminopenicillins, 4th generation cephalosporins, carbapenems, aminoglycosides, clindamycin, tetracyclines, glycopeptides, streptogramins, oxazolidones, pyrazinamide, rifamycins, chloramphenicol, metronidazole and polymyxin B.

The following classes of antimicrobials are classified as Important:

- 1st and 2nd generation cephalosporins, cephamycins, monobactams and quinolones,

The FDA website indicates that the guidance is under revision for the year 2019 [167].

5.11.4.4. FDA additional activities on AMR

In addition to the above guidance during the last years the FDA has produced relevant guidance restricting the number of antimicrobials that can be used as antimicrobial growth promoter (AGP) [168].

As detailed on the FDA Q&A document [169], the FDA provides guidance for pharmaceutical companies to voluntarily revise the FDA-approved labelled use conditions to remove the use of antimicrobial drugs for production purposes; add, where appropriate, scientifically-supported disease treatment, control or prevention uses; and change the marketing status from over-the-counter to Veterinary Feed Directive for drugs administered through feed or to prescription status for drugs administered through water in order to provide for veterinary oversight or consultation. In practical terms this means that companies were requested to modify the status to some of their AGP to VMPs, on which the FDA was very successful.

Once the legal status of a product is changed, it will be a violation of the Federal Food, Drug, and Cosmetic Act to use these products in feed for production purposes and would have to have a prescription or order from a licensed veterinarian to obtain these products.

As detailed in the mentioned Q&A document, one of the main differences between the FDA regulations and the EU New Veterinary Regulation is how the FDA treats the subject of preventive use of antimicrobials. The FDA indicates that for preventive use, veterinarians will need to consider if there is evidence that the antimicrobial will be effective in treating the particular disease, if the preventive use is consistent with accepted veterinary practice, if the use is intended to address particular bacteria, if the use is appropriately targeted to animals at risk of developing a specific disease, and if there are no reasonable alternatives for intervention.

It is encouraging to note that the 2017 FDA Summary Report On Antimicrobials Sold or Distributed for Use in Food-Producing Animals [170] highlights that: domestic sales and distribution of medically important antimicrobials approved for use in food-producing animals decreased by 33% from 2016 through 2017 and decreased by 43% from 2015 (the year of peak sales) through 2017.

5.12. Activities of other international organisations on AMR

5.12.1. TATFAR

The Transatlantic Taskforce on Antimicrobial Resistance (TATFAR), has its origins on the 2009 U.S. – EU Summit Declaration. Initially it was composed of experts from the EU and the US, later on, experts from Canada and Norway joined the task force.

The taskforce objectives for 2016-2020 as stated on their webpage [171] are to:

- Increase the mutual understanding of activities and programs relating to the prevention and control of AMR;
- Contribute to an effective global dialogue and uptake of best practices;
- Provide opportunities for shared learning; and
- Promote information exchange, coordination and cooperation between the TATFAR participating countries.

TATFAR has 3 main areas of collaboration:

- Improve appropriate therapeutic use of antimicrobial drugs in medical and veterinary communities,
- Prevent healthcare - and community-associated drug-resistant infections, and
- Develop strategies for improving the pipeline of new antimicrobial drugs.

In its progress report 2014 [172], one of the points to be addressed is that common measures of antimicrobial use in veterinary medicine are needed in order to compare data between the US and EU and to follow trends over time across sectors and regions.

TATFAR recommends collaborating on the collection of data on sales and use of veterinary antimicrobial drugs in food-producing animals. TATFAR is currently working on a proposal of data collection harmonisation. The experts working in antimicrobial consumption at TATFAR are highly skilful on the subject and it is to be hoped that a certain degree of harmonisation will be achieved during the next years.

5.12.2. Codex Alimentarius

The Codex Alimentarius is a collection of standards, guidelines and codes of practice established by FAO and WHO to protect consumer health and promote fair practices in food trade.

An Ad hoc Codex Intergovernmental Task Force on Antimicrobial Resistance (TFAMR) was created in 2007 to support the task of Codex Alimentarius on AMR.

The objective of the task force is to develop science-based guidance on the management of foodborne AMR, in line with the WHO Global Action Plan on Antimicrobial Resistance, the work and standards of relevant international organizations, such as FAO, WHO and OIE, following the One-Health approach. One of the main objectives of Codex in this area is to ensure that Members have the necessary guidance to enable coherent management of AMR along the food chain.

The terms of reference of the task force are to review and revise as appropriate the Code of Practice to Minimise and Contain Antimicrobial Resistance (CAC/RCP 61-2005) [173, 174] to address the entire food chain, in line with the mandate of Codex and to consider the development of Guidance on Integrated Surveillance of Antimicrobial Resistance, taking into account the guidance developed by the WHO Advisory Group on Integrated Surveillance of Antimicrobial Resistance (AGISAR) and relevant OIE documents.

The Codex task force on AMR shall complete its work by 2021.

The above-mentioned Code of Practice has provided a legal framework for worldwide countries to adapt regulations locally with the aim to diminish AMR related to food production.

5.12.3. CVMP Strategy on antimicrobials 2016 to 2020.

In November 2015 the CVMP published its strategy on antimicrobials 2016 to 2020 [48]. The strategy follows a series of previously published CVMP strategies on antimicrobials [47].

The vision of the CVMP strategy is the availability of effective antimicrobial medicines for the treatment of important infectious diseases of animals while also assuring minimum risks to animals or humans arising from their use.

The strategy itself does not propose actions at Member State level but should result in guidance that have an impact on the marketing authorisation of antimicrobials and finally on how those antimicrobials are authorised and used in the EU.

The strategy is of special importance for the implementation of prudent use measures and for signalling the direction of the CVMP, the EU scientific body on the authorisation of veterinary medicinal products. The strategy is a reflection of the EU on AMR and the use of antibiotics in animals.

It is not possible to quantify which is the impact of the CVMP strategy on the reduction of the use of antimicrobials in animals, especially since in many EU/EEA countries the antimicrobial use varies widely, therefore the strategy might have a different impact in different countries.

5.12.4. EC Guidelines for the prudent use of antimicrobials in veterinary medicine and actions taken by EU MSs

The EC Guidelines for the prudent use of antimicrobials in veterinary medicine were published in the EC Official Journal [46, 175].

The guidelines are a compilation of measures and recommendations from the EU MS on the use of antimicrobials in different animal productions and are aimed at the countries wanting to implement measures to promote prudent use or to improve those already in place. Those guidelines have been used as one of the sources of information to assess the impact of the measures taken during the EC fact-finding mission reports [46].

5.12.4.1. EC recommendations on prudent use and measures taken

The EC guidance contains recommendations on the use of CIAs, e.g. cephalosporins and fluoroquinolones, or recommendations on the off-label use of antimicrobials under the cascade, indicating that such use outside the marketing authorisations should be sufficiently justified and recorded.

The use of antimicrobials orally via feed and drinking water (which constitutes most of the use of antimicrobials in food-producing animals) is discouraged and individual treatments, e.g. injectables, preferred. Prophylaxis is discouraged, and the dose of orals products (and all antimicrobials in general) is to be monitored and recorded.

The homogeneity of the distribution of the antimicrobials in feed should be guaranteed (see chapter 9.3. of this report for further information).

It is recommended to introduce measures to limit financial incentives between veterinary practitioners, suppliers of antimicrobials and the pharmaceutical industry, and to restrict potential conflicts of interest, something that some EU MS have done with mixed results when measures have been taken in isolation and not as part of a plan that takes a holistic approach [32, 139].

The use of single substances instead of combinations of antimicrobials is recommended and importantly that all the substances in the combination are active against the target pathogens.

Taking samples and performing antimicrobial susceptibility testing on target pathogens is recommended, something of particular relevance for the use of CIAs.

The guidelines remark that disease prevention measures are paramount to reduce the need to reduce AMC of antimicrobials, however a detailed analysis of those are considered outside the scope of this document.

A summary of recommended measures is listed below:

- Implement hygiene and biosecurity measures, improving husbandry systems.
- Produce protocols for the prevention of infectious diseases, infection control and hygiene.
- Establish integrated production systems which avoid the need to buy and mix animal populations and to transport animals with unknown disease status.
- Avoid stressful situations which can weaken animals' immune systems.
- Establish herd-specific health plans to achieve a consistent improvement in herd health. Discourage health programmes in which animals are systematically treated with antimicrobials prophylactically.
- Implement programmes to control specific animal diseases by means of vaccination.
- Provide incentives to farmers to encourage them to adopt effective preventive measures to improve animal health and welfare standards.

The EC guidelines also include recommendations per animal species, which are not listed here. Those included e.g. the avoidance of systematic use of drying off in dairy cattle, or the development of vaccines for aquaculture use.

The main principles of those guidelines are listed in the Annexes of this document, followed by examples of actions taken by the MSs, or the CVMP, and considerations on those recommendations.

5.12.5. OECD

The OECD is an active player in the area of AMR due to the importance that the subject has on the economy.

Their web page on AMR [176] indicates that the OECD provides a forum for discussion and provides countries with the evidence to implement effective and cost-effective policies to tackle AMR, promote effective use of antimicrobials and incentivise research and development in the antibiotic sector.

The OECD estimates [177] that around 2.4 million people could die in Europe, North America and Australia between 2015-2050 due to the superbug infections unless more is done to stem antibiotic resistance. It also calculates that three out of four deaths could be averted by spending just 2 US dollars per person a year on measures as simple as handwashing and more prudent use of antimicrobials. This could be achieved by:

- Promoting better hygiene.
- Ending over prescription of antibiotics.
- Facilitating rapid testing for patients to determine whether they have a viral or bacterial infection.
- Avoiding delays in prescribing antibiotics.
- Mass media campaigns (on AMR).

On a report produced in 2015 in preparation of a G7 (Canada, France, Germany, Italy, Japan, the United Kingdom, and the United States) discussion [29] it is noted that at the global level, only 25% of countries have implemented a national policy to tackle AMR and less than 40% of countries have put in place infection prevention and control programmes for AMR. The report also highlights that patients infected by antimicrobial-resistant diseases are significantly more likely to develop complications (e.g. +13% limb loss and +71% complications in the central nervous system for infections by methicillin-resistant *S. Aureus*) and to die (e.g. up to 2-3 times higher mortality depending on the micro-organism).

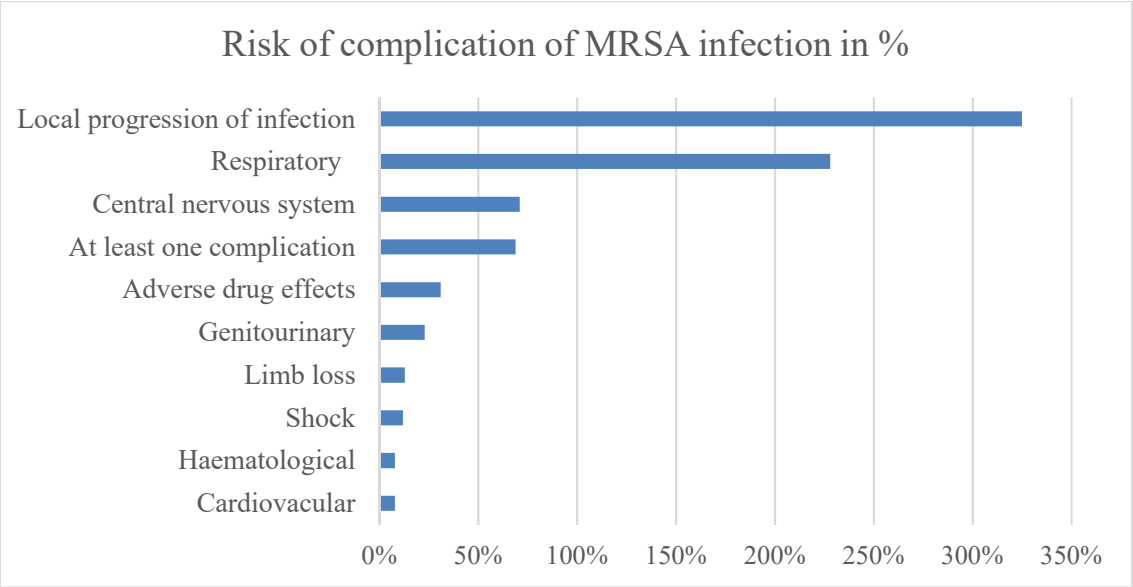


Figure 5. Additional risk of developing complications for infections by a resistant strain compared to a susceptible strain: the case of *S. aureus*. Adapted from OECD calculations [29] on Filice *et al.* [178].

The OECD notes that compared to a world with no AMR, the economic impact associated with current rates of AMR may reach about 0.03% of GDP in OECD countries in 2020, 0.07% in 2030 and 0.16% in 2050. This would result in cumulative losses of about US dollars 2.9 trillion [29].

In the above-mentioned report, the OECD highlights the reasons that AMR results in a more expensive patient treatment which include use of a more aggressive antimicrobial therapy based on either second-line antimicrobial treatments or combinations of different antimicrobial treatments.

Other reasons for more expensive treatments are that additional laboratory tests to ascertain which is the most effective therapy for a specific agent are required, as well as more intensive forms of treatments as, for instance, hospitalization in the case of community-acquired AMR micro-organisms or transfer to intensive care units and isolation rooms.

AMR infectious diseases require more intensive medical procedures including an increased likelihood of undergoing surgery among patients infected with resistant organisms are required as well as long stays at hospitals or treatment until the infection is eradicated.

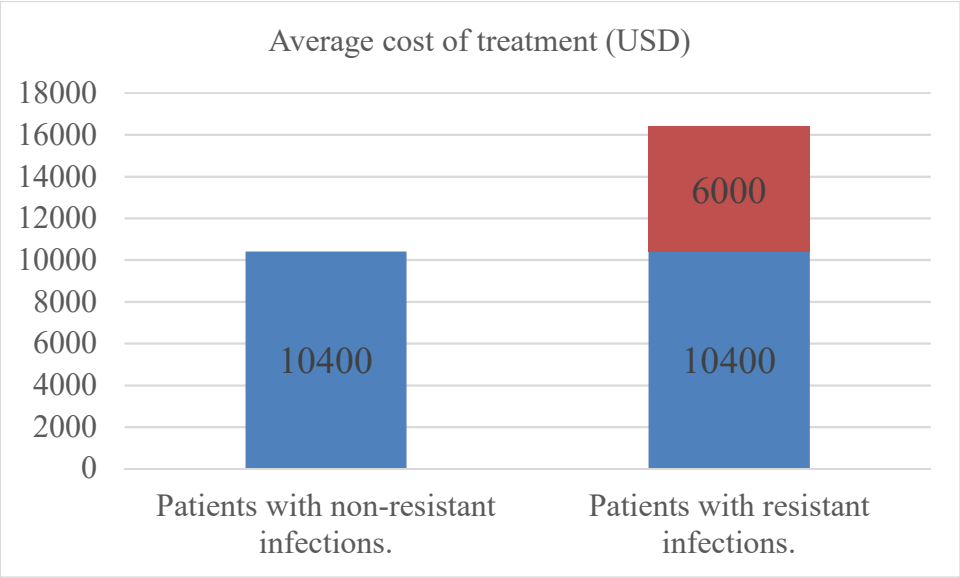


Figure 6. Costs of hospitalisation for patients with *E. coli* antibiotic-resistant infection and underlying drivers. Source: adapted from OECD analyses [177].

In addition to the above reports, the OECD has also recently produced a detailed report on antibiotic use and antibiotic resistance in food-producing animals in China [179].

Information about the use of antibiotics in animals in China has been scarce until recently, and even with some recent scientific publications [180, 181] and other reports with information on antimicrobial use in the country it is still difficult to pin the use of antimicrobials in animals in the country as the OECD report summarises very well.

In relation to the consumption of antimicrobials in mg/kg PCU, the report compiles data from different Chinese studies and reports reaching the conclusion that antibiotic use in China, as measured in terms of mg/PCU, is more than 5 times higher than the international average. And that animals in China consumed 51.5 mg/kg PCU in 2001 and 703 mg/kg PCU in 2007 [181].

The report also estimates that annual antimicrobial use (expressed as mg/kg PCU) in China in 2013 for pigs was 545 mg/kg PCU and 622 mg/kg PCU in broilers.

According to the report it is estimated that antimicrobial growth promoter (AGP) use accounts for roughly 60 to 70% of antimicrobials used by food-producing animals in China.

China is the largest producer and exporter of antimicrobials globally and accounts for approximately 70% of antimicrobials traded on international markets, which was to be expected. What is more remarkable is that China seem to account for about half of the global antimicrobial use in animal production, although the report also notes that its share may have fallen in recent years.

In China, antimicrobials are often used as an insurance to prevent an outbreak of animal disease that are associated with overcrowding and poor sanitation in pig and poultry production. The situation is further exacerbated as antimicrobials are widely regarded as an important growth stimulant. Currently 11 antimicrobials can be added to animal feed to enhance growth.

The report also refers to the lack of veterinary surveillance as one of the reasons for the high use of antimicrobials is that the number of rural veterinarians has fallen sharply in recent years as many vets chose to move into urban cities to meet the high demand to take care of pet animals. Sadly, the advisory role played by the vets is increasingly taken over by the salespersons of companies

that sell their products directly to the farmers, which according to the EU experience is leading to higher sales of antimicrobials.

The report notes that the main reason for the high antimicrobial use in animals is related to the misuse of antimicrobials, the widespread violation of government policies on antimicrobial use and misuse related to the lack of knowledge and skills in using antimicrobials

On a positive note the report also indicates that the Chinese Ministry has set policy targets to be reached by 2020, including to halve antimicrobial use in animal production by prescription; reduction of antibacterial use through the phasing out of antimicrobials that are important for human healthcare and those with potential of cross-transmission of AMR and antimicrobials used as AGPs, and to improve the monitoring system of antimicrobial use and AMR via enhancing technical standards and networking as well as educating users and veterinarians on the use of antimicrobials.

5.13. EMA recommendations on the impact of the use of antimicrobials in animals on public and animal health and measures to manage the possible risk to humans

As described above under 5.11.2. in 2013, the EC requested advice from the EMA on the impact of the use of antimicrobials in animals on public and animal health and measures to manage the possible risk to humans [165]. The request was divided into four questions and the first advice resulted in recommendations for colistin and tigecycline.

It is of relevance that the advice is the first advice from the CVMP (or CHMP) that links a recommendation to the reduction of the use of an antibiotic (colistin).

5.13.1. Tigecycline

Tigecycline is an interesting case of a substance not authorised for use in veterinary medicine (although there is some anecdotal evidence of use in dogs and cats) [182], however there is a high interest on the possible co-selection of resistance towards those crucial substances for human medicine from the use of substances of a similar class of antimicrobials in animals. This case is similar to carbapenamases [183].

The conclusion of the AMEG on tigecycline was that there is no available evidence of links between the use of tigecycline in animals and resistance to tigecycline. However, the recommendation also notes that tigecycline resistance might be impacted by the use of other antimicrobials (fluoroquinolones are cited), but such possible impact cannot be quantified. As a result the recommendation is that use of tigecycline in animals should be restricted and sets a series of stringent considerations that should be fulfilled to allow for the authorisation of tigecycline for use in veterinary medicine, the advice indicates is unlikely that the benefit-risk balance for such a product is going to be established as positive [182].

5.13.2. Colistin

On July 2013 the EMA/AMEG, following the above-mentioned request from the EC produced recommendations on the use of colistin in animals indicating that the use of colistin in animals was appropriate as long as some additional measures were taken [113].

Those additional measures were monitoring of off-label use, and restrictions on indications to therapy or metaphylaxis and removing all indications for prophylactic use with the aim to minimise any potential risk associated with high use of this substance in, e.g. health management programmes.

The initial colistin opinion indicated that for colistin use, detailed monitoring of colistin-resistant bacteria was required to confirm horizontal gene transfer is not involved, and that overall prevalence of resistance remains low. The initial opinion indicates that as soon as colistin resistance determinants are found on mobile genetic elements in the bacteria of concern as well as from human or animal origin, or a clonal explosion of virulent bacteria takes place, a new risk assessment would be required [113]. The last phrase of the assessment could be considered as premonitory of what was to happen.

On 18th of November of 2016, coinciding with the antibiotic awareness day, the detection of a colistin transferable gen; the *mcr-1* gen, would be published [184].

In human medicine and due to its systemic toxicity, colistin was used mostly for topical use, which resulted in low consumption [185], with limited use by other pharmaceutical forms due to problems with nephrotoxicity and neurotoxicity. The revised AMEG opinion [114] indicates that during the last 10 years, increasing numbers of hospital outbreaks with carbapenemase-producing Enterobacteriaceae *(E. coli, Klebsiella* species*),* and MDR *Pseudomonas* and *Acinetobacter* species (i.e. non-fermentative Gram-negative bacteria), have forced clinicians to reintroduce systemic colistin treatment, as a last resort drug for the treatment of healthcare-associated infections in which these organisms are involved.

Colistin has been used for decades in veterinary medicine with reported sales of 591 tonnes of polymyxins from 28 countries reporting to the ESVAC activity in 2014 [70]. In veterinary medicine, there are many products authorised for use in animals, mostly for oral administration [115, 116]. Most (if not all) the polymyxins used in the EU/EEA in animals are colistin [71].

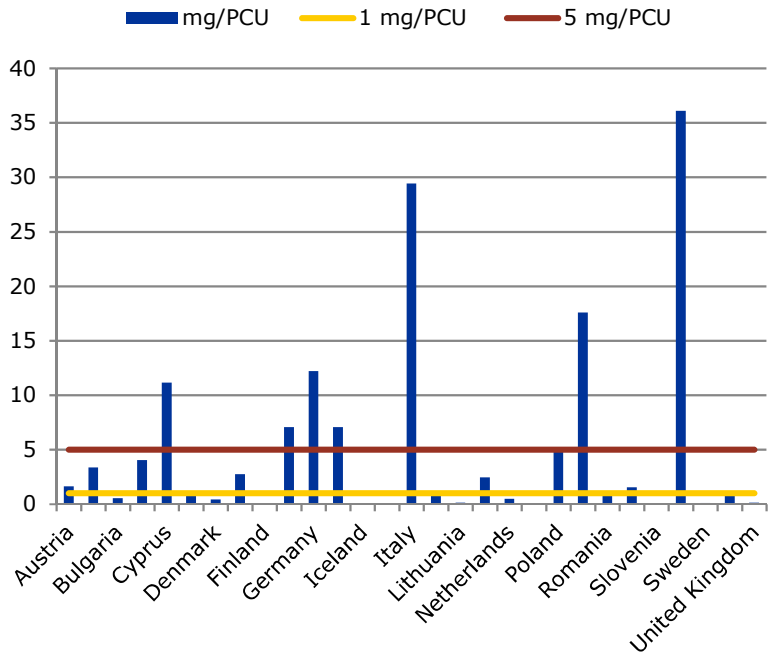


Figure 7. Sales of colistin for use in food-producing animals, in mg/PCU, in 2014, including the 5 and 1 mg/PCU levels (No sales in Finland, Iceland and Norway) [70].

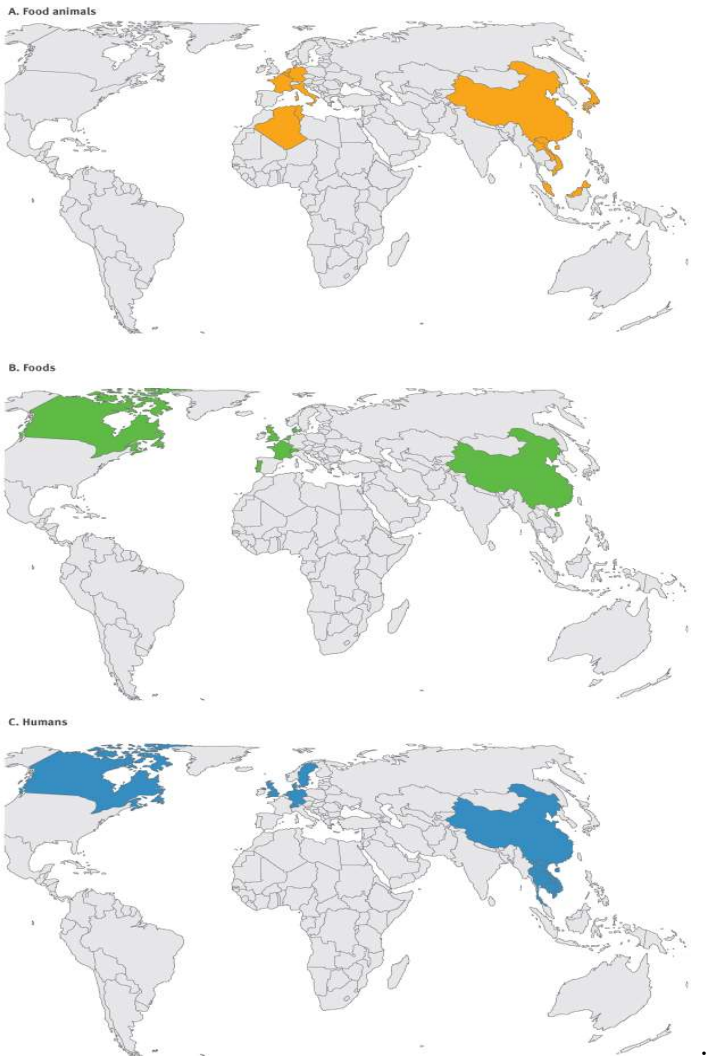


Figure 8. Maps of the worldwide geographic distribution of the *mcr*-1 gene in food animals, foods and humans [186].

Importantly, the newly discovered *mcr-1* gen suggests that colistin resistance has the potential to spread rapidly and to be associated with multidrug-resistant organisms, this could result in transfer to humans via different sources including food and water.

One of the most relevant conclusions of the revised AMEG opinion is that acquired resistance mechanisms are no longer limited to a stepwise process *via* mutations in target bacteria and plasmid-mediated spread is emerging, which is a departure from the previous understanding of the transmission of resistance in animals.

Resistance to other antimicrobial classes is frequently found in the same bacteria where the gen *mcr-1* has been found.

The main recommendation from the revised opinion is that the use of colistin in mg/PCU should be reduced. The recommendation was also that the four reductions should be achieved without an increase in the consumption of fluoroquinolones, 3rd and 4th generation cephalosporins or the overall use of antimicrobials.

No precise indication was provided on how such a reduction should be achieved.

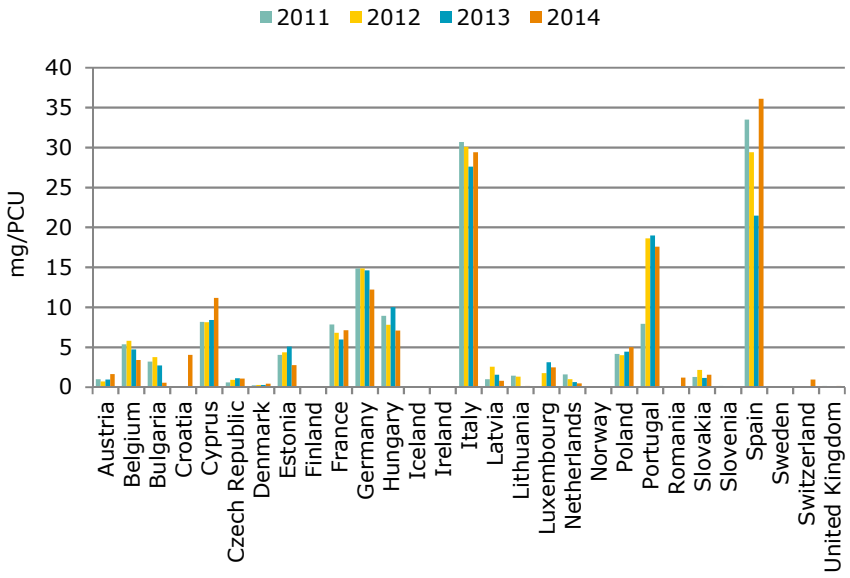


Figure 9. Sales of colistin for use in animals, in mg/PCU, in 29 European countries, from 2011 to 2014 (No sales in Finland, Iceland and Norway) [70].

The scientific advice, which is dated 2016 indicates that the targets for reduction should be achieved in a period of 3 to 4 years, which makes it more obvious the importance of the monitoring of the use of colistin in the EU.

5.14. EU indicators of AMR and AMC.

In 2016, the EC requested a joint ECDC, EFSA and EMA scientific opinion on a list of outcome indicators in regard to surveillance of AMR and AMC in humans and food-producing animals. The opinion was published in 2017 [187].

The terms of reference of the request were to jointly propose a list of outcome indicators suitable for monitoring and detecting reductions of relevant magnitude in the levels of key drug-resistant micro-organisms in humans, food-producing animals and food derived thereof and in AMC in humans and food-producing animal species.

The EC had specified that the Indicators should meet the following requirements: maximum of 15 indicators, divided in; four primary indicators and a maximum of 11 secondary indicators.

Those indicators should be used to assess progress made in MS AMR plans.

The indicators were to be suitable to estimate progress made in reducing AMR to key antimicrobials in accordance with WHO, AMEG and OIE definitions. They should also be robust, take into account a One Health approach to track and compare improvements made in human and veterinary sectors.

In addition, those indicators had to fulfil a series of conditions in relation to resistance: specify bacteria, population (human/animal), antimicrobial, recommended protocol, reporting unit.

And consumption: specify antimicrobial, sector (community/hospital), and reporting unit.

They were to be built where possible on data already collected and remain relevant for at least five years.

On the use of antimicrobials, the data for food-producing animals were provided by the EMA, and the data on human consumption of antimicrobials was provided by ECDC.

On AMR, the data on resistance in humans was provided by ECDC whilst the data on the resistance on food-producing animals and food thereof were provided by EFSA.

5.14.1. Indicators of AMC in humans (ECDC)

The agreed indicators were as follows:

Primary indicator:

- Total consumption of all antimicrobials for systemic use (DDD per 1,000 inhabitants and per day)

Secondary indicators:

- The ratio of consumption of broad-spectrum penicillins, cephalosporins, macrolides and fluoroquinolones to the consumption of narrow-spectrum penicillins, cephalosporins and macrolides;
- Consumption of glycopeptides, 3rd and 4th generation cephalosporins, monobactams, carbapenems, fluoroquinolones, polymyxins, piperacillin and enzyme inhibitors, linezolid, tedizolid and daptomycin (DDD per 1,000 inhabitants and day, and as a proportion of the total hospital use).

5.14.2. Indicators of AMR in humans (ECDC)

The agreed indicators were as follows:

Primary indicator:

- The proportion of methicillin-resistant *Staphylococcus aureus* (MRSA) and the proportion of *E. coli* resistant to 3rd generation cephalosporins (3rd generation cephalosporins resistant *E. coli*).

Secondary indicators:

- The proportion of *K. pneumoniae* isolates with combined resistance to aminoglycosides, fluoroquinolones and 3rd generation cephalosporins;
- The proportion of penicillin-resistant *S. pneumoniae* and proportion of macrolide-resistant *S. pneumoniae*;
- The proportion of carbapenem-resistant *K. pneumoniae*.

5.14.3. Indicators of AMR in food-producing animals (EFSA)

The agreed indicators were as follows:

Primary indicator:

- The proportion of indicator *E. coli* from broilers, fattening turkeys, fattening pigs and calves, weighted by PCU, fully susceptible to a predefined panel of antimicrobials.

Secondary indicators:

- The proportion of samples positive for presumptive ESBL-/AmpC-producing indicator *E. coli* from broilers, fattening turkeys, fattening pigs and calves weighted by PCU;
- The proportion of indicator *E. coli* from broilers, fattening turkeys, fattening pigs and calves, weighted by PCU, resistant to at least three antimicrobials from different classes included in a predefined panel of antimicrobials;
- The proportion of indicator *E. coli* from broilers, fattening turkeys, fattening pigs and calves, weighted by PCU, resistant to ciprofloxacin.

5.14.4. Indicators for AMC in food-producing animals (EMA)

The EMA indicators (indicators for AMC in food-producing animals) are those of relevance for this report.

Primary indicator:

- Overall sales of antimicrobials in mg/kg PCU

Secondary indicators:

- Sales of 3rd and 4th generation cephalosporins (mg/kg PCU)
- Sales of all quinolones, specifying the % of fluoroquinolones and quinolones (mg/kg PCU)
- Sales of polymyxins (mg/kg PCU)

The ESVAC/EMA publishes on its web page the results of those indicators, for the countries for which the data are available [188].

Table 2. Percentages of sales for food-producing animals (including horses), in mg per population correction unit (mg/PCU), of the various veterinary antimicrobial classes, by country, for 2016.

Country	Sales in mg per PCU				Proportion (%) fluoroquinolones *vs* all quinolones
	Overall sales	3rd -4th gen. cephalos-porins	Polymyxins	All quinolones (fluoroquinolones + other quinolones)	
Austria	46.10	0.22	1.6	0.51	100%
Belgium	140.14	0.30	2.4	0.94	62%
Bulgaria	155.26	0.10	2.2	5.23	93%
Croatia	87.92	0.16	3.5	3.10	85%
Cyprus	453.41	0.70	11.1	2.12	75%
Czech Republic	61.24	0.41	< 1mg/PCU	1.70	98%
Denmark	40.77	0.01	< 1mg/PCU	0.37	1%
Estonia	64.01	0.73	< 1mg/PCU	1.29	100%
Finland	18.59	0.01	NS	0.15	100%
France	71.94	0.06	2.7	0.67	33%
Germany	89.21	0.38	7.8	1.02	100%
Greece	63.50	0.10	1	6.98	32%
Hungary	187.05	0.42	12.2	9.79	98%
Iceland	4.66	0.00	NS	0.01	100%
Ireland	52.13	0.13	< 1mg/PCU	0.48	100%
Italy	294.77	0.38	15	4.75	49%
Latvia	29.91	0.26	< 1mg/PCU	0.85	99%
Lithuania	37.67	0.13	< 1mg/PCU	1.05	94%
Luxembourg	35.52	0.73	1	0.83	98%
Netherlands	52.74	0.00	< 1mg/PCU	0.98	9%

Norway	2.93	0.00	NS	0.04	14%
Poland	129.37	0.16	5.6	9.69	100%
Portugal	207.97	0.46	13.5	9.01	99%
Romania	85.13	0.08	5.5	3.48	94%
Slovakia	50.41	0.36	1.1	3.66	99%
Slovenia	30.31	0.16	< 1mg/PCU	2.95	99%
Spain	362.46	0.30	22	9.29	92%
Sweden	12.14	0.00	< 1mg/PCU	0.07	33%
Switzerland	46.64	0.16	< 1mg/PCU	0.35	100%
United Kingdom	39.33	0.14	< 1mg/PCU	0.23	100%

NS: No sales
Tablets are excluded from the sales data for food-producing animals as they are assumed to be used almost solely in companion animals.

5.14.5. Summary of the ECDC, EFSA and EMA Joint Scientific Opinion on a list of outcome indicators as regards surveillance of AMR and AMC in humans and food-producing animals

The scientific opinion includes a series of recommendations which are summarised below:

- The chosen indicators should be reconsidered at least every five years to evaluate whether they still reflect the data available.
- Data on resistance should be monitored on a continuous basis, in order to follow current AMR issues.
- Indicators in the different sectors should be analysed together within a MS.
- Data on AMC in animals should in the future be collected at farm level and according to different production systems. Analysis should take into account differences in dosing between species and substances, e.g. using the DDDvet system.
- Management decisions should never be based on these indicators alone and during evaluation of the effectiveness of any national intervention care must be taken to use appropriate statistical techniques.

The ECDC, EFSA and EMA Joint Scientific Opinion list of outcome indicators in regards to surveillance of AMR and AMC in humans and food-producing animals was published in 2017 on the EFSA Journal [187]. Considering that one of the recommendations of the report is that those indicators should be revised every five years, by 2022 a new revision of the indicators should be produced.

Main objective of the thesis

6. Main objective of the thesis

The thesis attempts to answer the detailed hypothesis analysing the sales and trends of antimicrobials for use in animals in the EU/EEA and Switzerland during the years 2010 to 2016, measuring if there has been a reduction in AMC in animals during those years, and more specifically:

- Analysing the sales and trends of antimicrobials for use in animals in the EU/EEA, and Switzerland, during the years 2010 to 2016.
- Assessing whether there has been a reduction in AMC in animals during those years (in total and for some classes of antimicrobials).
- Studying the correlations between the use of antimicrobials and various parameters such as:
    - The use of some pharmaceutical forms.
    - Data collection by animal species.
    - AMC early monitoring.
    - Types of animal production.
    - Temperature.

Hypothesis

7. Hypothesis

1. Between 2010 and 2016, the use of antimicrobials in animals in the EU/EEA has been reduced in a statistically significant manner.
2. A: Between 2010 and 2016 the sales of some of the HPCIA classes of antimicrobials has been reduced in a statistically significant manner. Those classes of antimicrobials are 3rd and 4th generation cephalosporins, quinolones and polymyxins.

   B: Sales of some of the HPCIA classes of antimicrobials in 2016 correlates with the overall sales of antimicrobials in the country in 2016.
3. The use of pharmaceutical forms for group treatment is correlated with high overall sales of antimicrobials.
4. Collecting data on antimicrobial use per animal species is correlated with an overall decrease of antimicrobials.
5. Collecting data on antimicrobial sales before 2007 (pre-ESVAC) in a country is correlated with low sales of antimicrobials in 2016 in the country.
6. The production of the main animal species (pigs, poultry and cattle) is correlated to the overall mg/kg PCU in 2016.
7. The average temperature in a country is correlated with the overall use of antimicrobials in animals in the country in 2016.

Material and methods

8. Material and methods

The main material of this thesis is the sales data of antimicrobials for use in animals collected, evaluated and published by ESVAC [63-65, 70, 71, 189], especially of some of those considered highly critically important by the WHO (3rd and 4th generation cephalosporins, quinolones and polymyxins) [155], and those in Category B (Restrict) of the AMEG [9, 10] between the years 2010 and 2016 (both inclusive).

The data on AMC are analysed against the production in weight of food-producing species in the country every year (e.g. pigs, cattle and poultry).

ESVAC sales data of antimicrobials for use in animals are provided annually to the EMA, which validates those data [61-65, 70, 71, 74, 189-192].

The Agency annually produces an estimated animal biomass of the weight of the animals at treatment; the population correction unit (PCU). This data following its collection and analysis are published annually.

Data are available since 2010, although not for all the countries, for this reason, some of the data for the analysis (e.g. decrease of sales) are from 2011 (Bulgaria, Cyprus, Germany, Poland and Slovakia) or in one case (Luxembourg) from 2012. Those data were compared with the data from the latest year available; i.e. the year 2016.

The data analysed are:

- Decrease of the overall AMC divided by the estimated animal biomass at treatment (mg/kg PCU) between the years 2010 and 2016 in the countries participating in the ESVAC project (hypothesis 1).
- The decrease in the consumption of 3rd and 4th generation cephalosporins, quinolones and polymyxins, expressed as mg/kg PCU between 2010 and 2016 (hypothesis 2A).
- Correlation of the consumption of 3rd and 4th generation cephalosporins, quinolones and polymyxins, expressed as mg/kg PCU between 2010 and 2016 with the overall sales of antimicrobials in the year 2016 (hypothesis 2B).
- Correlation of the proportion of use of pharmaceutical forms for group treatment with the overall sales of antimicrobials in animals in mg/kg PCU (year 2016) (hypothesis 3).
- Correlation of the AMC expressed as mg/kg PCU in the countries collecting data by animal species and those not collecting data per animal species (hypothesis 4) in 2016.
- Correlation between the existence of a system to collect sales data before 2007 (pre-ESVAC) and the overall sales of antimicrobials in animals in mg/kg PCU (year 2016) of those countries versus those not collecting antimicrobial sales data in animals (hypothesis 5).
- Correlation of the proportion of species of animals intensively farmed (pigs plus poultry), as well as use in pigs, poultry and cattle (year 2016) with the overall sales of antimicrobials for antimicrobial use animals in mg/kg PCU (year 2016) (hypothesis 6).
- Correlation of the antimicrobial sales for animal use (expressed as mg/kg PCU) in 2016 with the average temperature of a country in 2016 (hypothesis 7).

- The setting of a quantifiable target to reduce AMC in animals at national level *vs* overall sales of antimicrobials in animals in mg/kg PCU (year 2015). This hypothesis was later disregarded.
- The existence of a contract between the farmer and the veterinarian and the AMC of antimicrobials in mg/kg PCU (no statistical analysis produced). This hypothesis was later disregarded.

Scientific databases (PUBMED, Library of Congress) were searched by the terms: Country *(each of the EU/EEA countries)* (antibiotic or antimicrobial) (livestock or animals or cattle or pig or poultry or broiler or horse or fish) for further information on reports of studies analysing AMC. However, due to resources limitations, it was not possible to perform a strict literature search [193, 194].

8.1. Data from ESVAC for the analysis

The variables reported to ESVAC for each antimicrobial veterinary medicinal product are described in detail in the ESVAC reports [61-65, 70, 189], the most relevant details of those data can be found in 12.5. Annex V – Scope of ESVAC data.

In the ESVAC project, for antimicrobial veterinary medicinal products containing more than one active ingredient, information on the active ingredient name, strength and strength unit must be given for each ingredient separately.

Critically the ESVAC data is composed of the sales of each package of each antimicrobial VMP which allows the ESVAC project to validate those data by e.g. comparing the data with the data provided the previous years.

As an example, the ESVAC report from 2016, includes data from 9,205 product presentations (tablets excluded) [71].

8.1.1. The PCU

The estimated animal biomass to which the animals can be exposed to antimicrobials is a crucial parameter to put into context the antimicrobials sold in a population, otherwise it is not possible to analyse the use of antimicrobials against the animal population which could theoretically be exposed to the use of antimicrobials, e.g. countries with a large animal population would be penalised against those with a small animal population as the latter case would have a small overall AMC compared to the large AMC in the bigger country, even if the country with a large population would be using fewer antibiotics per kg of animal produced.

The estimated animal biomass is usually referred to as the “denominator” where the “numerator” [73, 77, 187] would refer to the antimicrobials consumed by a specific animal population (e.g. a farm holding or a country)

In order to contextualise the use of antimicrobials in, e.g. a country or a farm, the concept of the PCU was introduced by the ESVAC project.

From the beginning of the project, the limitations of the estimated animal biomass were identified, indicating that sales cannot be reported relative to the size of the target animal population.

A pragmatic approach was used to estimate the total biomass of major production animals as the denominator. Which include data from e.g. slaughtered pigs, poultry and cattle and of (live) dairy cattle [192, 195], this resulted in the Population Correction Unit (PCU) which has been used since then by the ESVAC project which is described below [61-64, 70, 73, 189-191, 196].

Lately there has been some criticism of the PCU methodology, especially in what refers to the estimated animal weight at treatment [197], resulting in some countries creating an adapted PCU, which in the case of the mentioned publication is the adjusted population correction unit, which resulted in some important increases in the estimated animal population in 8 European countries and Canada; i.e. increase in cattle biomass (35% to 43%), whilst the biomass of pig and poultry decreased by approximately 51% and 87% respectively. Such criticism is also shared by at least the Dutch SDa, personal communication, [198]). The model followed by the SDa is based on the average kilogram present, which, according to the SDa, represents the average animal weight at risk of being treated with antibiotics [198, 199].

Although the above considerations merit further reflection, the PCU methodology has been adopted by many countries and institutions in order to express the sales of antimicrobials for use in animals [67-69, 197, 200-203].

Table 3. The animal categories included in the calculation of the PCU and data types to be reported is an extensive list which includes:

Cattle (heads)	Pigs (heads)	Poultry (heads)	Caprinae (heads)	*Equidae*, rabbits (heads) & fish (tonnes)
Slaughtered cows Slaughtered heifers Slaughtered bullocks and bulls Slaughtered calves and young cattle Import slaughter Export slaughter Import fatteners Export fatteners Living dairy cows	Slaughtered pigs Import slaughter Export slaughter Import fatteners Export fatteners Living sows	Slaughtered broilers Slaughtered turkeys Import slaughter Export slaughter	Slaughtered sheep and goats Import sheep slaughter Export sheep slaughter Import sheep fatteners Export sheep fatteners Living sheep Import goats slaughter Export goats slaughter Import goats fatteners Export goats fatteners	Living horses Slaughtered rabbits Biomass fish slaughter weight

The weights used to calculate the population correction unit which are collected from guidelines on environmental risk assessment based on Montforts [90] are detailed in one of the Annexes of this report.

8.2. Indicators analysed for the report

The indicators used for the analysis are:

8.2.1. Quantitative indicators

- Sales of all antimicrobials for use in food-producing species per country expressed as mg/kg PCU.
- Variation of overall sales in mg/kg PCU (estimated animal biomass).
- Sales of 3rd and 4th generation cephalosporins for use in food-producing species per country expressed as mg/kg PCU.
- Variation of mg/kg PCU 3rd and 4th generation cephalosporins.
- Sales of quinolones for use in food-producing species per country expressed as mg/kg PCU.
- Variation of mg/kg PCU of all quinolones.
- Sales of polymyxins for use in food-producing species per country expressed as mg/kg PCU.
- Decrease of mg/kg PCU of polymyxins.
- The proportion of use of group treatment pharmaceutical forms versus sales of all pharmaceutical forms.
- The proportion of each of the pharmaceutical forms for group treatment *vs* sales of all pharmaceutical forms.
- The proportion of intensive farming (pigs and poultry) *vs* total animals produced.
- The proportion of each of the major animal producing species (cattle, pigs and poultry) *vs* total animals produced.
- The average temperature in a country *vs* AMC.
- The average high temperature, average low temperature and average precipitation *vs* AMC.

8.2.2. Qualitative indicators

- The collection of data on the use of antimicrobials by animal species in the year 2016.
- The collection of data on the use of antimicrobials before the year 2007 (pre-ESVAC).

8.2.3. Justification for the chosen indicators:

8.2.3.1. Decrease of the overall sales in mg/kg PCU (estimated animal biomass)

The mg of antimicrobials sold for animals per kg of PCU per year is the main indicator of the ESVAC project and has been agreed by the EMA, ECDC and EFSA [204]. It is an indicator that has now been adopted by some other countries (Canada, Japan…) [201, 202] and reflects the overall sales by animal biomass without distinction of the animal species in which the antimicrobial is used or the class of antimicrobial.

The OIE has also adapted the ESVAC collection of data [67-69]; however the OIE animal biomass differs from that of the ESVAC as detailed under 5.6.1.

8.2.3.2. Decrease of the mg/kg PCU of 3rd and 4th generation cephalosporins.

The mg of 3rd and 4th generation cephalosporins sold to animals per kg of PCU per year is a secondary indicator of the ESVAC project [71, 187, 204].

Third and 4th generation cephalosporins are HPCIA according to the WHO [155-160] and the highest category of the AMEG classification [9, 10]. The OIE does also make specific recommendations for the use of those antimicrobials [161, 162].

8.2.3.3. Decrease of the mg/kg PCU of all quinolones.

The milligrams of quinolones sold to animals per kg of PCU per year is a secondary indicator of the ESVAC project [187, 204].

Quinolones are HPCIA according to the WHO [155-159]. Quinolones are part of the highest category of the AMEG classification [9, 10].

The OIE does also make specific recommendations for the use of those antimicrobials, although it refers to the group of fluoroquinolones, i.e. excluding the quinolones that are not fluoroquinolones, namely oxolinic acid and flumequine [161, 162].

8.2.3.4. Decrease of the mg/kg PCU of polymyxins.

The mg of polymyxins sold to animals per kg of PCU per year is a secondary indicator of the ESVAC project [187, 204].

Polymyxins are HPCIA according to the WHO [155] and have been recently included in the highest category of the AMEG [10]. The OIE has also recently made changes on its recommendations to include polymyxins on their lists of classes of antimicrobials that require special consideration.

Polymyxins use in veterinary medicine is mostly composed of the substance colistin [114] with some use of polymyxin B. However, during the preparation of the latest ESVAC report, it was accounted that all the sales of polymyxins refer to the substance colistin [71].

8.2.3.5. The proportion of use of antimicrobials for group treatment versus all pharmaceutical forms

This indicator is of interest to analyse the association between the uses of antimicrobials in group treatments versus individual treatments. It is assumed that less use of group treatments in favour of individual treatments would be associated with less use of antimicrobials.

In some countries the use of antimicrobials is mostly by injections, this is especially visible in the Nordic countries where the proportion of injectables used is higher than in the rest of the EU.

National legislation, traditions or even the products available might have an impact on the pharmaceutical forms sold; this is very obvious in the case of Germany where there are hardly any sales of premixes (see Table 17) but where some of those might have been substituted by sales of other oral forms. There are some limitations on the analysis of those data that are discussed under Chapter 9.3. Results of sales of antimicrobials (as mg/kg PCU) of oral forms for group treatment.

8.2.3.6. Collection of data on use of antimicrobials by animal species

The existence of schemas to collect data on the use of antimicrobials by animal species has been analysed against the overall sales.

Collection of use data (data collected at e.g. farms or by prescription) could provide an indication of not only the willingness of a country to survey and analyse the use of antimicrobials in animals but also the disposition to promote prudent use of those antimicrobials and is expected to be linked to a reduction of sales.

In the analysis of the collection of AMC per animal species (e.g. poultry or pigs), the analysis of the results is difficult as many countries are implementing projects for the collection of data per animal species, but with a limited scope. Some countries are limiting such collection to e.g. pigs (or pigs and poultry) [88].

The ESVAC project had an ongoing plan/strategy to collect and publish EU data from animal species at farm level. As a first step data was collected from pig farms (test of the trial) [196] but the project did not gain continuity due to the cost of the collection of such data, and also the lack of legal requirements for such collection.

The publication of Regulation (EU) 2019 [1], that includes many tools to allow for the collection of data at farm level has changed this situation, providing a legal requirement for the collection of data by animal species.

The Regulation means that by 2030 the EU MSs should be collecting data on antimicrobial use of nearly all the animal species; companion and food-producing species. No other area/region in the world has set such an ambitious project in relation to the collection of data on consumption of antimicrobials in animals.

8.2.3.7. Collection of data of antimicrobials for use in animals before ESVAC

When the ESVAC project was started back in 2009, only a few countries collected data on AMC in the EU, many of those countries were Nordic countries. It is difficult to set clear cut criteria of when and which countries started to collect data on AMC.

The initial work of ESVAC focussed on producing a harmonised publication of the data from those countries [195].

Some countries might at the time have had some partial collection of data or even an overall collection of data that were not published. In order to set objective criteria, the publication from Grave 2010 [195] has been used as the reference publication.

At the time of the start of the ESVAC project (year 2009), a thorough analysis of the countries already collecting data of AMC for animal use was performed, and it can be concluded that those countries selected were the countries that were collecting and publishing those data in a regular manner. According to the mentioned publication, those countries were: Czech Republic, Denmark, Finland, France, Germany, the Netherlands, Norway, Sweden, Switzerland and the United Kingdom. The data used in the publication are mostly from 2007, except for Germany in which case the data were from 2005.

In order to simplify the reference to the data the time for the collection of data is compiled as: Collecting data on antimicrobial sales before 2007 (pre-ESVAC).

A correlation between low sales of antimicrobials and collecting data pre-ESVAC could indicate an early willingness from a country to take action on the problems related to the use of antimicrobials in animals for public health.

8.2.3.8. The animal species produced is associated with the overall sales of antimicrobials

The use of antimicrobials in animals in a country will vary depending on the animal species produced [139]. Countries with more intensive production of animals will consume more antimicrobials per kg produced of animal [139, 196].

The correlation between the sales of antimicrobials in 2016 *vs* the animal production in each country was analysed. To do such an analysis, the animal production collected as PCU was used.

Unfortunately, the PCU has limitations for this analysis as the data collected do not distinguish between which animals are grown in, e.g. free-range conditions or which are kept in intensive conditions. This is especially problematic for cattle where some of its production can be extensive, but some of it can be very intensive with high consumption of antimicrobials (e.g. feedlots) [89].

The sum of the production of pigs and poultry, two of the most intensively produced species [89, 179] was analysed, as well as the productions of pigs, poultry, cattle and other species separately.

8.2.3.9. The average temperature in a country vs the overall use of antimicrobials in animals in the country in 2016.

Although a circumstantial factor, it is not uncommon to indicate that higher temperatures in a country can favour more use of antimicrobials as hot weather would result in more bacterial infections. McFadden [205] found that increasing local temperature, as well as population density, are associated with increasing antibiotic resistance (percent resistant) in common pathogens (in humans), with increases in antibiotic resistance of 4.2%, 2.2%, and 2.7% for *Escherichia coli, Klebsiella pneumoniae and Staphylococcus aureus*. The publication also highlights that European countries in the southern latitudes have a higher incidence of infections due to the ESBL-producing *Enterobacteriaceae*, which has generally been attributed to antibiotic use and selective pressure, but which may be facilitated by climate factors, including temperature.

The estimated correlation between AMC and temperature could be criticised as it points to a very general factor; the average temperature of a country, something that is obviously composed of very heterogeneous data from different areas (e.g. the average temperature within different areas of Italy or Spain might vary importantly). Nevertheless, considering the recent publication and the impact that temperature has on raising animals, and farming conditions, is a parameter that was deemed worth considering.

8.2.4. Justification for disregarding some of the indicators:

Some indicators were rejected to be analysed for the following reasons:

- **The existence of a contract between veterinarians and farmers and its association with sales of antimicrobials**.

  Those contracts oblige farmers to have a contract with a veterinarian allowing those to more freely choose the prescription of antimicrobials. This is the case for Denmark [206] and the Netherlands [198, 207]. However, due to the low number of countries with such type of contracts a statistical analysis of those data were not considered necessary; these countries have

a low level of use of antimicrobials (Denmark), or a high reduction in the use of antimicrobials (the Netherlands) or a combination of both (see Table 4).

- **Targets on antimicrobial use reduction.**

  With this qualitative indicator, there was an intention to analyse if the setting of a target antimicrobial use reduction has resulted in a reduction of AMC in the country.

  Setting targets is considered one of the most effective measures on reducing the need for the use of antimicrobials [139, 208, 209]. However due to the difficulty in establishing which species restrictions had been introduced, and when those restrictions had been implemented, and if those had been compulsory or voluntary, it resulted in a complexity that could not be addressed during this thesis. Nevertheless, the quantitative study of the impact of the implementation of targets for antimicrobial reduction remains as one of the areas of most interest to identify how to reduce AMC in animals.

- **Guidelines on prudent use of antimicrobials.**

  The existence of prudent use guidelines of antimicrobials for use in animals *vs* the use of antimicrobials in a country was analysed.

  The publication of national plans on AMR on the web was studied, including the existence of dedicated guidance by animal species using sources such as the MSs reports, the EC fact-finding missions, as well as other EC relevant reports [21, 210, 211], and the ESVAC reports. The WHO directory of AMR action plans [23, 33, 66] was considered, as well as any reference available from the publicly available literature.

  However, this indicator was disregarded due to the difficulty of setting an objective criteria selection on when, who and under which conditions those guidelines were applied (e.g. animal species, the target of the guidelines, compliance follow up).

- **The economic benefits of veterinarians from sales of antimicrobials**.

  This indicator was disregarded due to the difficulties of setting objective criteria selection of what can be interpreted as *"economic benefits of veterinarians from sales of antimicrobials"*, not only based on the legal prohibition but also on the need to assess the measures to implement those prohibitions.

  There have been intensive discussions about the decoupling of sales of antimicrobials and its prescription [139, 208].

  The sales of some countries where sales of antimicrobials by veterinarians are permitted (e.g. France [127]) have been reduced enormously during the last years [212], whilst other countries where sales of antimicrobials are not allowed by veterinarians have not reduced sales, or those remain as high consumers of antimicrobials, e.g. the case of Spain [65, 123].

- **The percentage of use of CIAs versus total use of antimicrobials**

  The indicator was considered and consequently disregarded.

  The reason for disregarding this indicator on the % of the use of CIAs is that some of the CIAs are used at small doses (e.g. fluoroquinolones or cephalosporins) compared to other substances like tetracyclines which require much higher doses (in mg/kg antimicrobial) per treatment. Without data that take into account the potency and dosification of antimicrobials such as DDDvet (or DCDvet), in order to estimate the % of CIAs weight data on, e.g. cephalosporins with macrolides would have had to be added and compared with the weight of non-CIAs, which would have distorted the whole analysis.

Due to the lack of collection of data by species it is not possible to collect those data as DDDvet (or DCDvet), in other words, the % would have analysed the accumulated sales of CIAs but was not taking into account the different dosing of those substances, a problem that also occurs for the ESVAC report but that was more relevant for this indicator, and for this reason the indicator was disregarded.

8.3. Statistical analysis

The statistical tests used to address the hypotheses were:

- t-Student test (paired or unpaired, as convenient) - or U Mann-Whitney or T Wilcoxon in case of not normal distribution - were employed to contrast differences or the reduction of use or sales of antimicrobials in hypothesis 2A, 4 and 5.

  To assess normality, tests of Kolmogorov-Smirnov were performed.

- Pearson´s linear correlation for the rest of the hypothesis, using the categorisation from Evans [213], which is categorised as follows depending on the results:
  - 0-0.19 "very weak."
  - 0.20-0.39 "weak."
  - 0.40-0.59 "moderate."
  - 0.60-0.79 "strong."
  - 0.80-1 "very strong"
- Curve Estimation procedure were performed to compare the linear and exponential relationship between indicators when necessary.

Values of $p < 0.05$ were considered significant. All statistical analysis was performed using IBM Statistics, version 25 (SPSS).

8.3.1. Identification of outliers

When the interquartile range is above 1.5, those values are identified as outliers and are presented as a circle by the SPPS software. When the interquartile range is greater than 3, the values are considered extreme values and are presented as an asterisk.

According to Hoaglin and Iglewicz [214], qualifying values of 1.5 times the interquartile as outliers might be inaccurate in about 50% of the times, and suggested that the outliers should be those where the values are at least 2.2 times the interquartile values. However, SPSS only expresses the outliers as 1.5 times (^{o}) or as 3 times (*) the interquartile value, and as such were considered as outliers.

Results

9. Results

The data on sales of antimicrobials were obtained from the ESVAC project and its database. Those results are publicly available from the EMA/ESVAC web page, note that the online tool has different sheets [188] where different information is provided.

Some of the data were not available online and were obtained from the ESVAC reports [61-65, 70, 71, 189] and not from the database, this is the case for some data for the year 2010, and some of the data for Switzerland.

When comparing the latest available data from, e.g. 2016 with those of 2010 to analyse trends data for Bulgaria, Cyprus, Germany, Poland and Slovakia were only available from the year 2011.

Data for Lithuania, the Netherlands and Spain were not available for the year 2010 on the ESVAC database but were available from the printed ESVAC reports, however some of those data, e.g. mg/kg PCU for quinolones, 3rd and 4th generation cephalosporins and polymyxins were excluded from the analysis by classes of antimicrobials, due to the lack of detail on the ESVAC report.

Data for Luxembourg were available from 2012 and were also incorporated into the trend analysis.

Data from Switzerland were not provided at package level to the ESVAC project until 2014, and for this reason, are not included in the ESVAC database [70]. However, those data are analysed and reported on the ESVAC reports and have been included in the analysis when available. Sales for “other quinolones” were added to “other antimicrobials” for confidentiality reasons for years 2010, 2011, 2012 and 2013 so sales of quinolones could be an underestimate for those years and were disregarded for the trend analysis.

Data for Croatia, Greece and Romania were not obtained until after the year 2013 and have not been incorporated into the trend analysis.

Data for Malta, the only EU country for which data were not available, were not available for 2016 but are likely to be available for the report of data in 2017.

9.1. Results of overall sales of antimicrobials

9.1.1. Results of overall sales of antimicrobials in mg/kg PCU

Table 4. Overall sales of antimicrobials in mg/kg PCU, for the years 2010 to 2016 including the variation in the % of sales between the years 2010 and 2016*, in 30 European countries, where available§.

Country	mg/kg PCU							Percentage overall sales mg/kg PCU, years 2010* to 2016
	2010	2011	2012	2013	2014	2015	2016	
Austria	62.9	54.5	54.9	57.2	56.3	50.7	46.1	-26.7%
Belgium	180.1	175.3	163.1	156.6	158.3	150.1	140.1	-22.2%
Bulgaria	NA	92.6	98.9	116.1	82.9	121.9	155.3	***67.7%***
Croatia	NA				108.6	95.6	87.9	Not computed
Cyprus	NA	***407.6***	***396.5***	***425.8***	***391.5***	***434.2***	***453.4***	11.2%
Czech Republic	94.3	83.0	79.8	82.2	79.5	68.1	61.2	-35.1%
Denmark	47.5	42.6	44.1	44.9	44.2	42.2	40.8	-14.1%
Estonia	70.9	70.7	62.9	70.4	77.1	65.2	64.0	-9.7%
Finland	22.7	21.9	21.8	22.4	22.3	20.4	18.6	-18.1%
France	136.0	116.5	102.7	95.0	107.0	70.2	71.9	-47.1%
Germany	NA	211.5	204.8	179.7	149.3	97.9	89.2	-57.8%
Greece	NA					57.2	63.5	Not computed
Hungary	269.9	192.5	245.8	230.7	193.1	211.4	187.1	-30.7%
Iceland	7.3	6.6	5.9	5.3	5.2	5.0	4.7	-35.6%
Ireland	51.5	46.5	55.0	55.9	47.6	51.0	52.1	1.2%

Country	mg/kg PCU							Percentage overall sales mg/kg PCU, years 2010* to 2016
	2010	2011	2012	2013	2014	2015	2016	
Italy	***421.1***	371.0	341.0	301.6	332.4	322.0	294.8	-30.0%
Latvia	39.5	36.7	41.5	37.7	36.7	37.6	29.9	-24.3%
Lithuania	48.2	41.3	39.2	29.1	35.5	35.1	37.7	-21.8%
Luxembourg	NA		43.2	52.1	40.9	34.6	35.5	-17.8%
Netherlands	146.2	113.8	74.9	69.9	68.4	64.4	52.7	***-64.0%***
Norway	***4.1***	***3.7***	***3.8***	***3.7***	***3.1***	***2.9***	***2.9***	-29.3%
Poland	NA	127.3	135.2	151.5	140.8	138.9	129.4	1.6%
Portugal	177.9	161.8	156.9	187.2	201.6	134.4	208.0	16.9%
Romania	NA				109.0	100.5	85.2	Not computed
Slovakia	NA	43.7	43.3	63.1	65.9	53.8	50.4	15.3%
Slovenia	46.9	46.1	37.0	22.4	33.4	26.4	30.3	-35.4%
Spain	259.5	335.8	302.4	317.1	418.8	402.0	362.5	39.7%
Sweden	15.2	13.6	13.5	12.7	11.5	11.8	12.1	-20.4%
Switzerland	78.9	78.9	68.8	64.5	56.9	50.6	46.6	-40.9%
United Kingdom	67.9	51.1	66.3	62.1	62.1	56.7	45.0	-33.7%

*For some countries (Croatia, Greece and Romania) data were not collected until after the year 2013, and the % of change was not computed. For Bulgaria, Cyprus, Germany, Poland and Slovakia the data are from the year 2011, and for Luxembourg, 2012.

§ NA: Not available

Blue, bold and italics highlight the lower result or % value per column. Red, bold and italics the highest result or % per column.

The lowest sales of antimicrobials per mg/kg PCU were for Norway (years 2010 to 2016), and the highest for Italy (year 2010) and Cyprus (years 2011 to 2016). The biggest decrease of antimicrobials sold per mg/kg PCU was for the Netherlands (-64.0%, years 2010 to 2016), and the highest increase for Bulgaria (67.7%, years 2011 to 2016).

Table 5. Overall sales statistics in mg/kg PCU in the years 2010 to 2016.

		Overall sales in mg/kg PCU*						
		2010	2011	2012	2013	2014	2015	2016
Valid		21	26	27	27	29	30	30
Missing*		9	4	3	3	1	0	0
Mean		107.1	113.3	107.6	107.9	108.3	101.5	98.6
Std. Deviation		105.2	111.0	105.1	105.9	108.2	109.1	107.0
Minimum		4.0	3.6	3.8	3.6	3.1	2.9	2.9
Maximum		421.1	407.6	396.5	425.8	418.8	434.2	453.4
Percentiles	25	43.2	42.3	41.5	37.7	38.8	37.0	37.1
	50	67.9	74.8	66.3	64.5	68.4	60.8	57.0
	75	162.0	165.2	156.9	156.6	145.0	126.2	132.1

*Countries not included by year:
2010: Bulgaria, Croatia, Cyprus, Germany, Greece, Luxembourg, Poland, Romania, Slovakia.
2011: Croatia, Greece, Luxembourg and Romania.
2012: Croatia, Greece and Romania.
2013: Croatia, Greece and Romania.
2014: Greece.

For the % overall sales mg/kg PCU, years 2010 (2011 or 2012) (117.6) to 2016 (98.6) the mean was a decrease in sales of 17.1% (standard deviation 28.7%).

9.1.2. Overall sales mean in mg/kg PCU: two alternatives

The mean of the calculated mg/kg PCU can be made based on the addition of the previously calculated mg/kg PCU of each country and diving it by the number of countries:

$$Mean\ (mg/PCU) = \frac{mg/PCU_{Country}}{Number\ of\ countries} \qquad \text{(Equation 1)}$$

where considering that each country has the same portion of the mg/kg PCU or by adding the total tonnes of antimicrobials and divide it by the total animal biomass.

$$Mean\ (mg/\text{kg}\ PCU) = \frac{Total\ tonnes\ of\ antimicrobials\ sold\ in\ all\ countries}{Animal\ biomass\ in\ all\ participating\ countries} \qquad \text{(Equation 2)}$$

The table below shows the results of the mean of total sales calculated by both criteria for the years 2010 to 2016, both inclusive.

Table 6. Comparison of the mg/kg PCU calculated as the addition of total sales divided by animal production *vs* average of mg/kg PCU during the years 2010 to 2016.

	Overall sales in mg/kg PCU						
	2010	2011	2012	2013	2014	2015	2016
mg/kg PCU calculated by dividing the mg/kg PCU per country by the number of countries (eq. 1)	107.1	113.3	107.6	107.9	108.3	101.5	98.6
Addition of total sales of antimicrobials divided by animal production (eq. 2)	NA*	162.0	152.4	146.9	156.2	140.8	129.4
Percentage of difference between the two methods of estimation		30.1	29.4	26.5	30.7	27.9	23.8

*Not available

As can be observed in Table 6 the differences between the estimation of the mg/kg PCU calculated by dividing the mg/kg PCU per country by the number of countries and calculated as the addition of total sales of antimicrobials divided by animal production is substantial.

The mean of total sales, in mg/kg PCU, calculated by equation (1) is lower than that obtained with equation 2, by an average of 28.1%. The difference is caused by the bigger consumption of antimicrobials in big countries (e.g. Spain, Italy, France and Germany), which increases the mg/kg PCU, and the smaller consumption in some of the small countries (e.g. Iceland, Norway, Sweden and Finland), when compared to the average of each country.

However, those differences remain relatively constant through the years of the estimations.

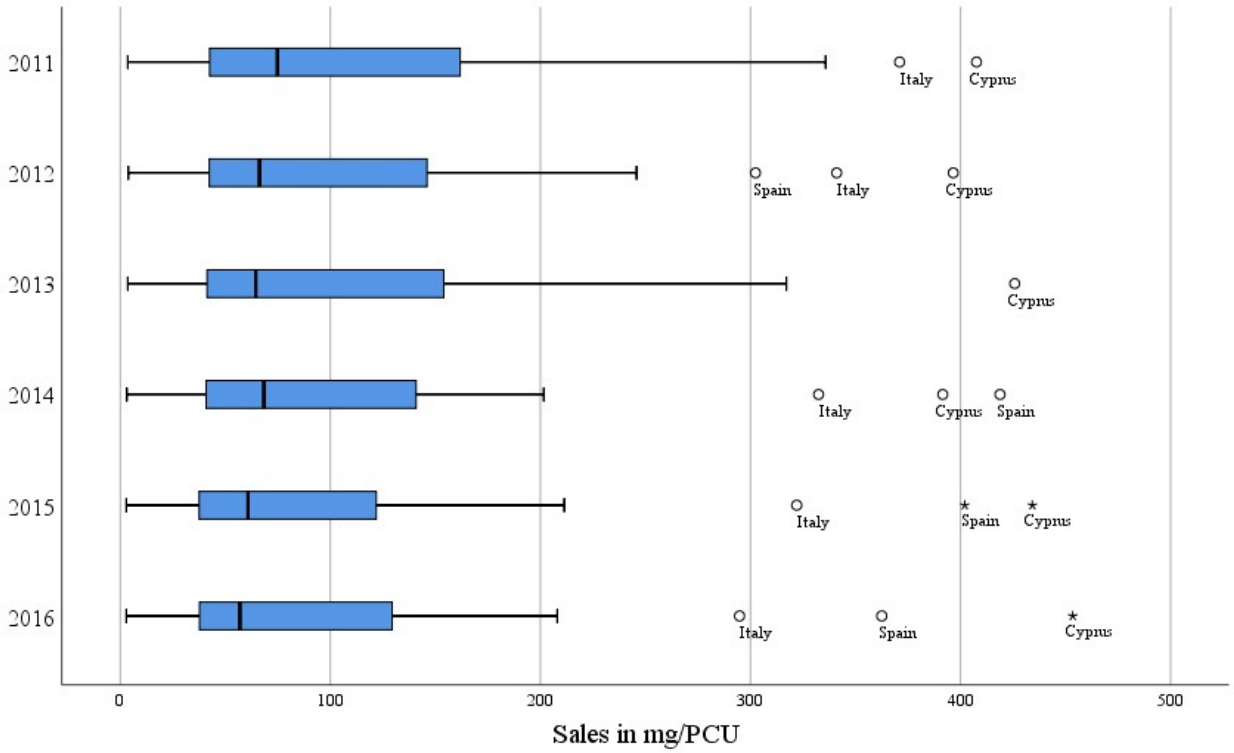


Figure 10. Overall sales in mg/kg PCU in for the years 2010 – 2016. The years in the graph are ordered in increasing order, with data from 2016 at the bottom of the graph.

Sales for Italy, Spain and Cyprus were above the 1.5 times interquartile for most of the years.

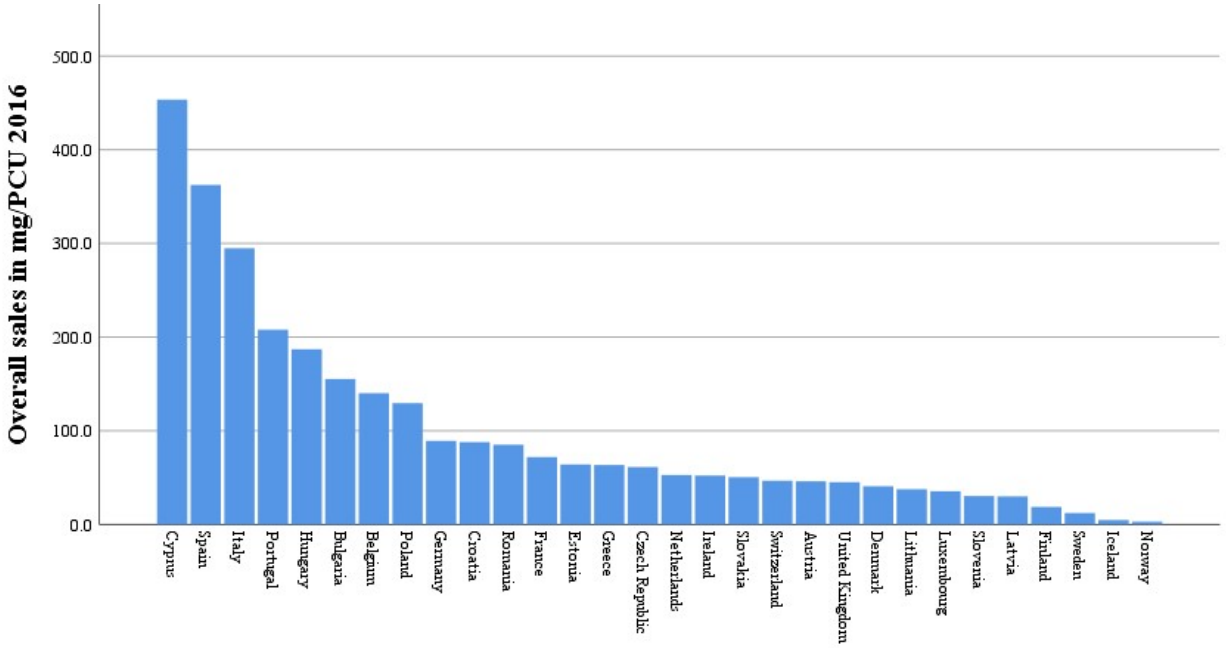


Figure 11. Overall sales of antimicrobials in mg/kg PCU, in order of sales of antimicrobials in mg/kg PCU for the year 2016 (the last year for which data are available), in decreasing order.

The data show a striking difference between the sales of antimicrobials for use in animals in the country selling the most mg of antimicrobials per kg of animal produced (Cyprus, 453,4 mg/kg PCU) *vs* the country with the least sales of antimicrobials per kg of animal produced (Norway, 2,9 mg/kg PCU). The difference in the use of antimicrobials between both countries is greater than 150 times.

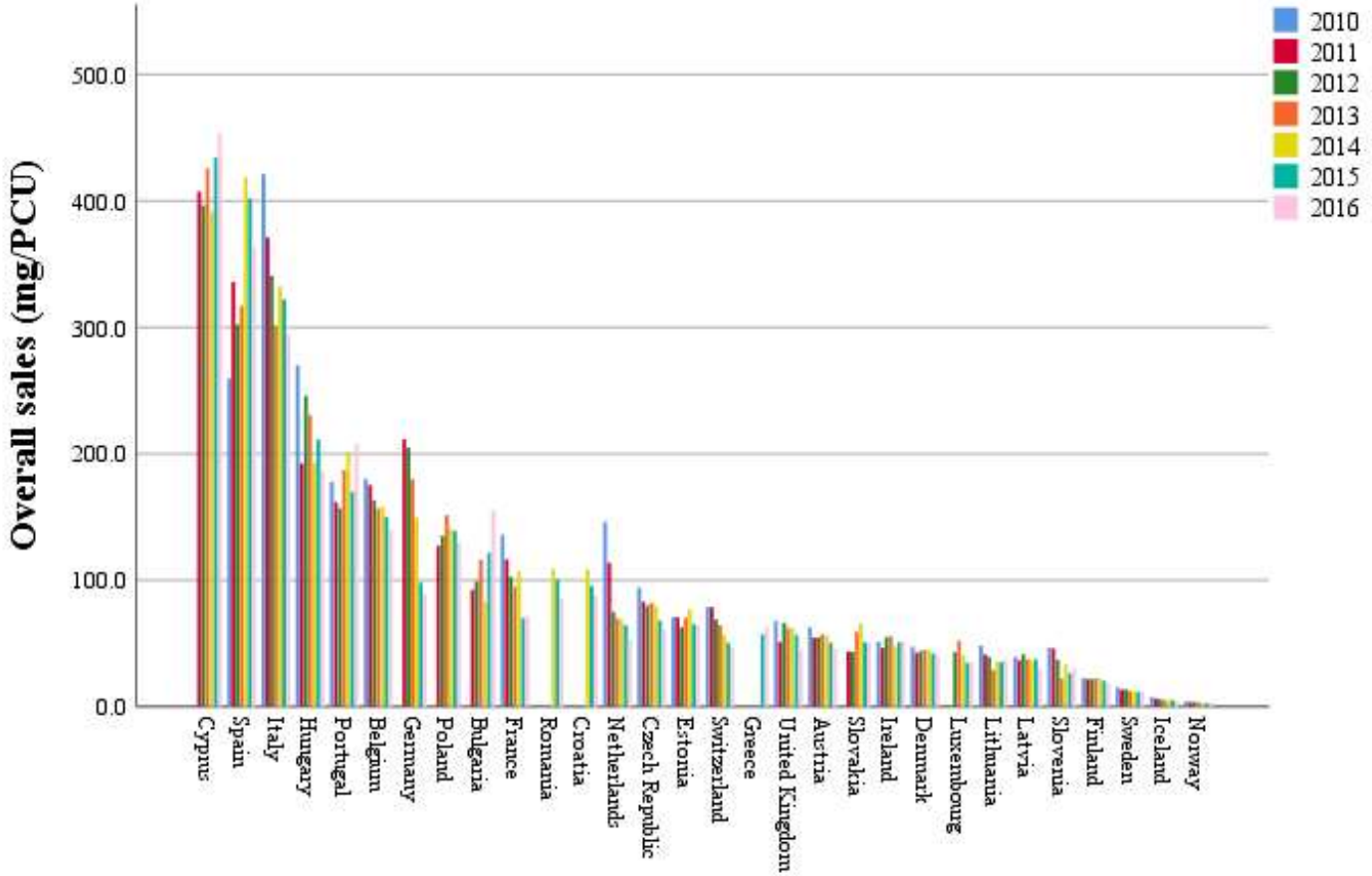


Figure 12. Overall sales of antimicrobials for all years (2010 to 2016) and all countries reporting data to ESVAC in mg/kg PCU.

The following three figures, Figure 13 to Figure 15, show the sales in mg/kg PCU for the years 2010 to 2016, where available, considering 3 groups: low, middle and high; according to their sales in 2016.

In the figures, the countries are shown in ascending order of sales and attention should be paid to the different scales in them.

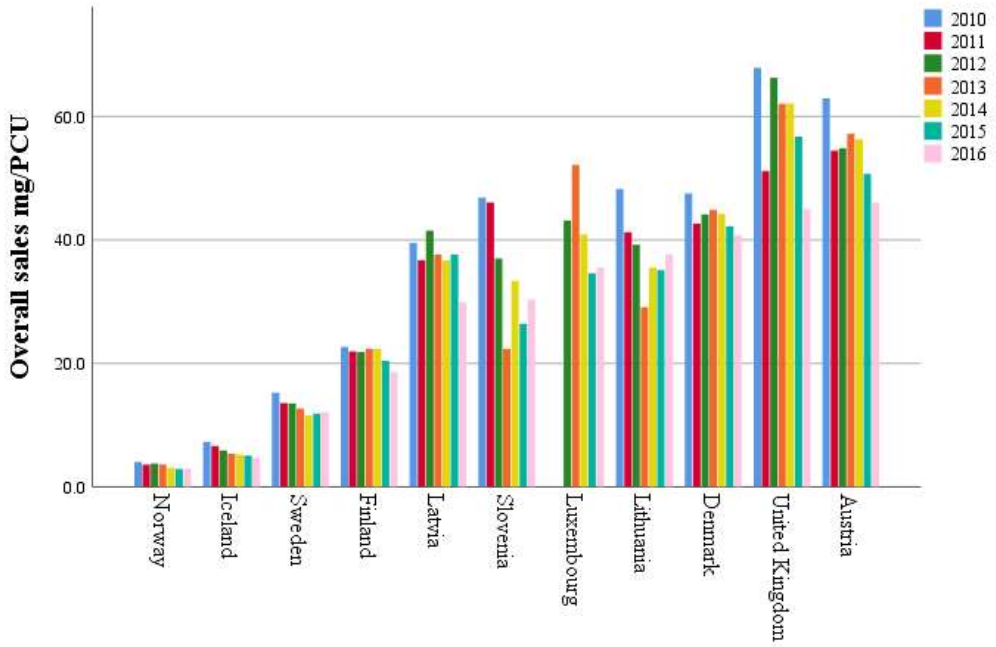


Figure 13. Overall sales of antimicrobials in mg/kg PCU – ***low countries consumption*** for all years (2010 to 2016).

All the countries in the low consumption group have continued decreasing the sales of antimicrobials for use in food-producing species, this might be the result of their constant campaigns to reduce antimicrobial use in animals and multifactorial approach to the problem of AMR [32].

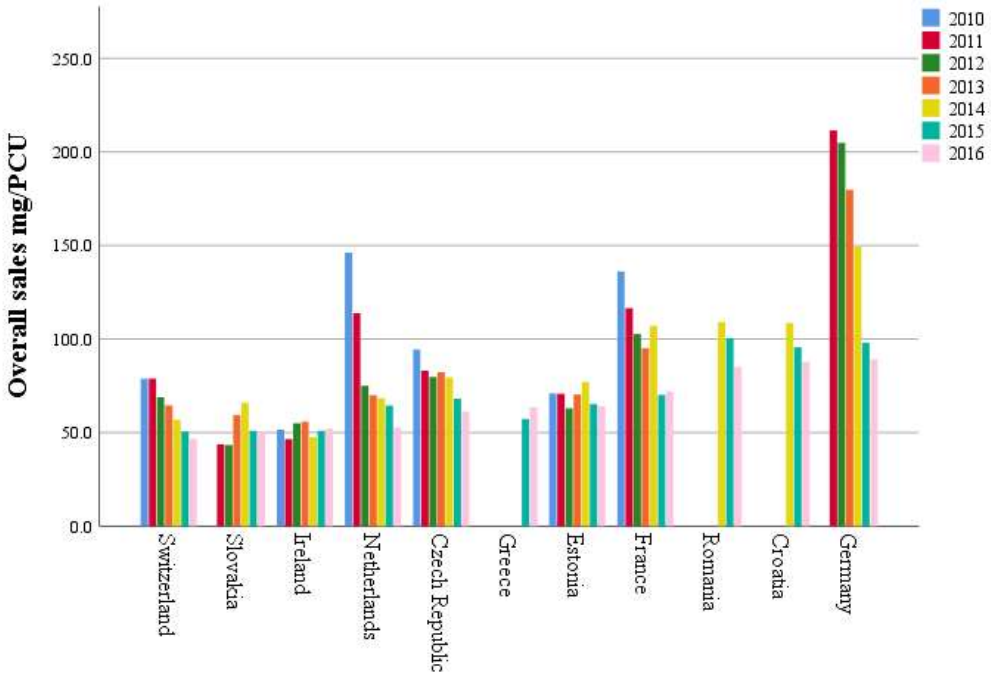


Figure 14. Overall sales of antimicrobials in mg/kg PCU – ***middle countries consumption*** for all years (2010 to 2016).

In the middle group only Slovakia and Greece (although data are only available for two years for Greece), have increased their sales.

The reduction in overall sales of antimicrobials in countries like the Netherlands, France and Germany are striking.

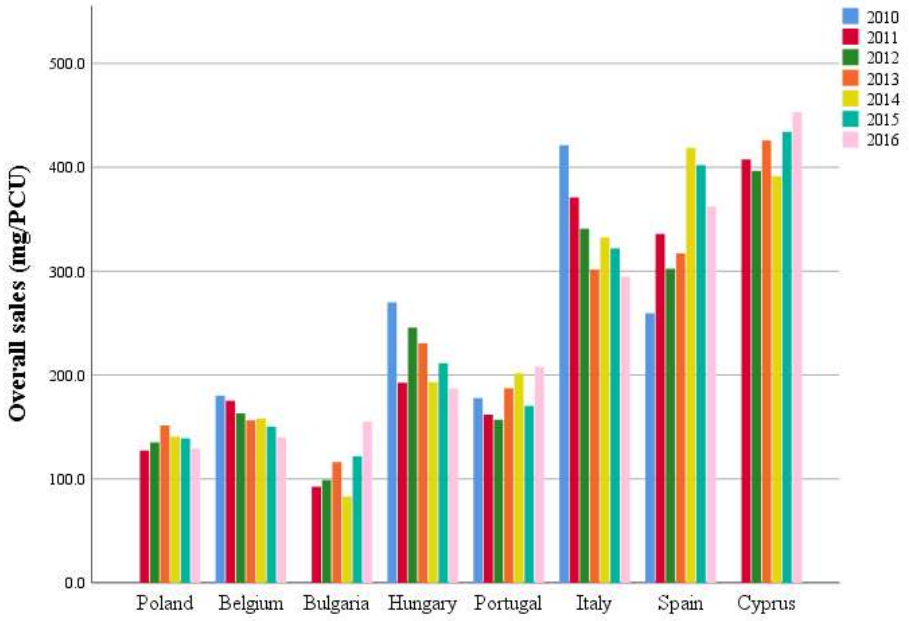


Figure 15. Overall sales of antimicrobials in mg/kg PCU – ***high countries consumption*** for all years (2010 to 2016).

Of the high users' group, only Belgium, Hungary and Italy show a decrease on their sales, whilst Poland, Bulgaria, Portugal, Spain and Cyprus show a high increase on sales between the years 2010 and 2016.

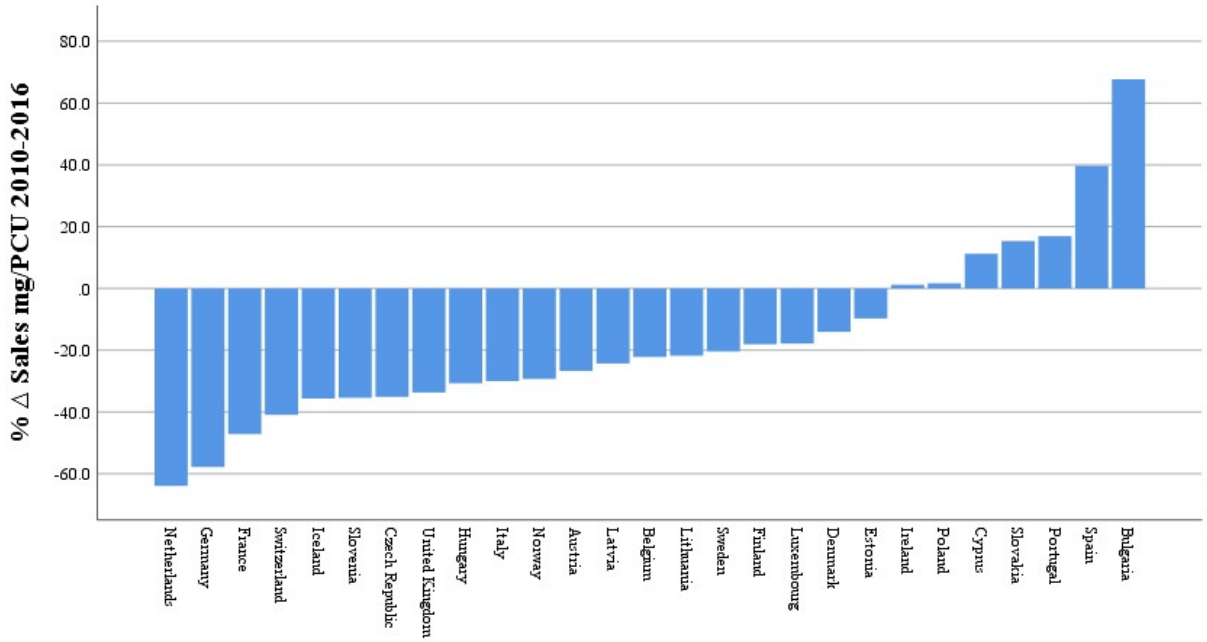


Figure 16. Variation on the percentage of overall sales of antimicrobials in mg/kg PCU, for the years 2010 (2011 or 2012) and 2016, for 27 countries in increasing order.

The majority of countries show a sharp decrease in antimicrobial use (see Figure 16).

The mean decrease in sales is 17.1%, the median 22.2%, and the standard deviation of 28.7%. As described in the ESVAC reports, there have been some changes in data collection from Bulgaria and Spain that question the reported increase in those two countries. For Spain, the ESVAC report with data collected in 2014 [70] indicates that Spain changed its system for collecting sales data in 2014, and there were indications that some of the highest-selling veterinary medicinal products for 2014 had not been reported by MAHs between 2011–2013 despite being marketed during this period. Therefore, the suggestion is that the sales data for Spain from 2011 to 2013 represent substantial underestimates.

The ESVAC report with data collected in 2013 [189] indicates that for Bulgaria and Slovakia, no conclusion can be made on whether there has been an increase or decrease in sales due to under-reporting for 2011 and 2012.

Both countries (Bulgaria and Slovakia) reported sales of antimicrobials for animal use in the year 2011 for the first time, so from the described under-reporting during the initial years of data collection it can be assumed that the supposed increased of sales on the period 2011-2016 for Bulgaria (67.7%) and Slovakia (15.3%) are overestimates.

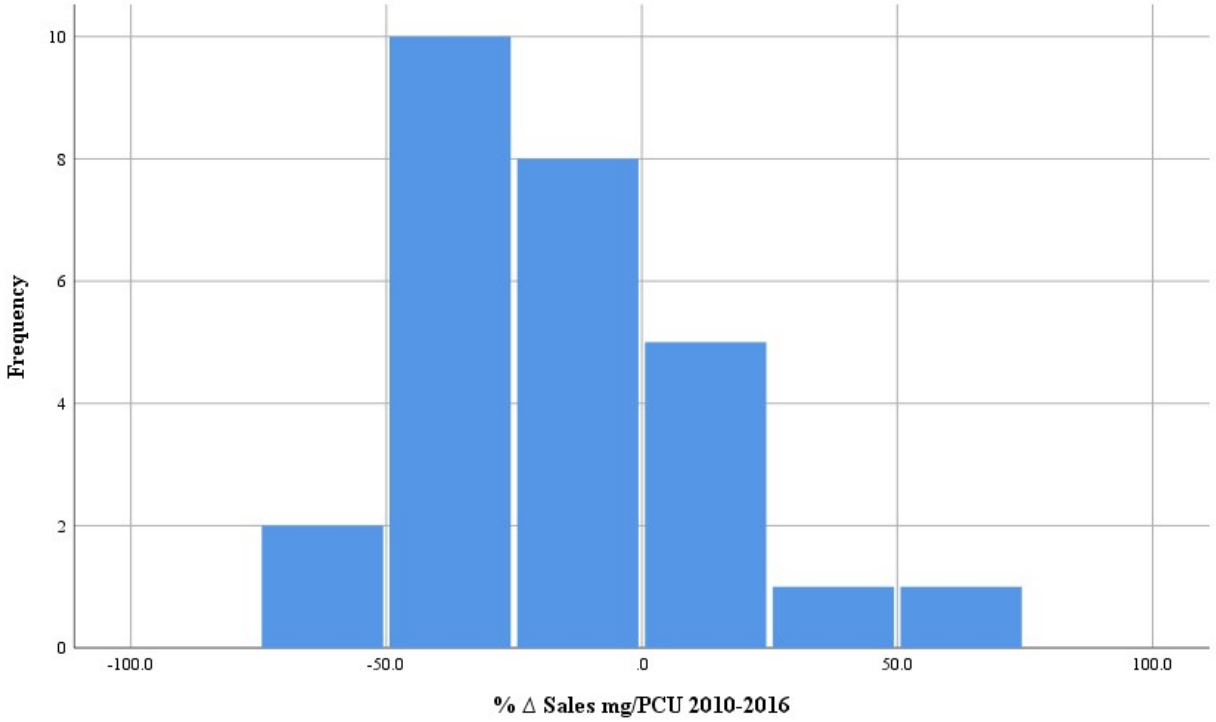


Figure 17. Variation on the percentage of overall sales of antimicrobials in mg/kg PCU, for the years 2010 - 2016.

Twenty countries had a decrease in sales of antimicrobials in mg/kg PCU in the period 2010 (2011 or 2012) to 2016, whilst 7 countries had an increase on sales of antimicrobials in the same period.

The decrease in sales of antimicrobials for food-producing species for 27 countries, between 2010 and 2016 are statistically significant (< 0.005, one-tailed).

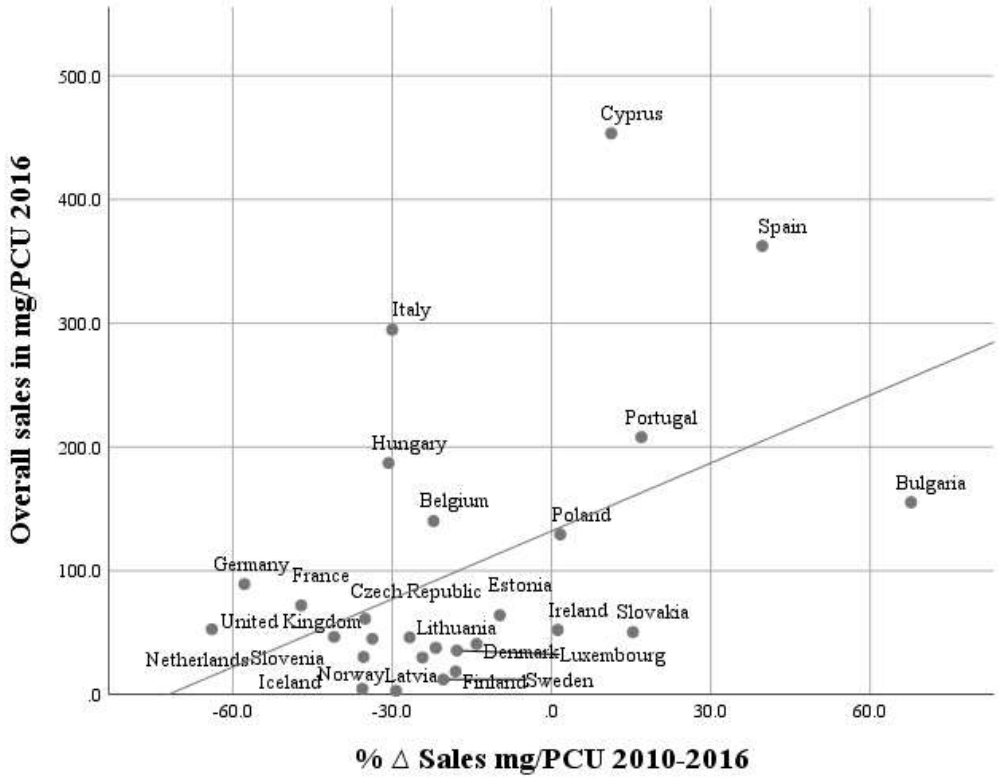


Figure 18. Variation in percentage on mg/kg PCU between 2010 (or 2011 or 2012) and 2016 *vs* overall sales in mg/kg PCU in 2016.

The Pearson's linear correlation between the overall sales of antimicrobials in 2016 and the variation of sales between 2010 and 2016 is moderate (0.467) and significative at the 0.05 level but not at the cut-off point of the Bonferroni correction (see 9.8. for more details).

The above Figure 18 shows a big dispersion of results between those countries with high antibiotic consumption in 2016 with respect to the variation of their sales during the period 2010 to 2016.

Some countries like Bulgaria and Spain have increased their sales, instead of what could be expected (a decrease) following EU policies to decrease AMC, the EC fact-finding reports for those countries provide justification for such increase [123, 144].

Other countries like Portugal and Cyprus have remained with sales similar to previous years. The decrease of AMC in some countries like Italy and Hungary is also remarkable although they remain in the group of high consumers of antimicrobials.

The decrease of the overall sales of antimicrobials in the Netherlands, France and Germany is truly remarkable, especially since all of them are countries with a big animal production, which results in a higher decrease of total antimicrobial use per year, and all of them are now below 100 mg/kg PCU.

It is also remarkable that all countries that already had a low consumption have continued reducing their AMC (Iceland, -35.6%, Norway -29.3%, Sweden, -20.4%, Finland, -18.1% and Denmark, -14.1%).

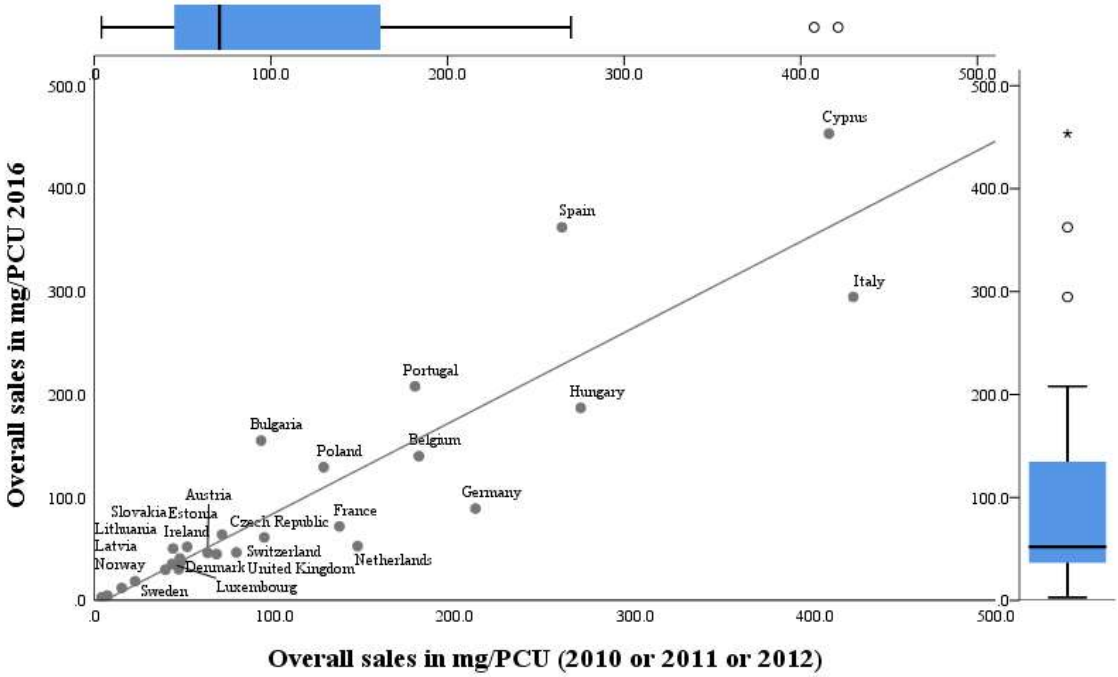


Figure 19. Sales of all antimicrobials in mg/kg PCU in 2010 (or 2011 or 2012) *vs* overall sales in 2016.

The Pearson's linear correlation of sales of all antimicrobials in mg/kg PCU in 2010 (or 2011 or 2012) *vs* overall sales in 2016 is very strong (0.900) and significant with the Bonferroni correction (<0.00147 level, 2-tailed) (see 9.8.).

Although the sales of some countries like Italy have been reduced notably (-30%), as the initial sales were very high (421.1 mg PCU for Italy in 2010), the mg/kg PCU is still one of the highest of the ESVAC reporting countries.

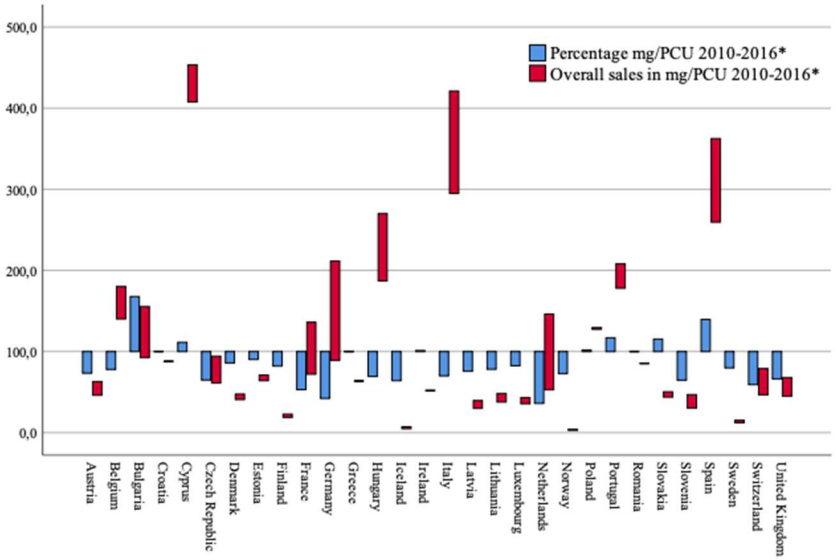


Figure 20. Variation of the means in sales of all antimicrobials between 2010 and 2016 in mg/kg PCU.
*Every country shows 2 bars: * red range bars is the variation of sales in mg/kg PCU between 2016 and 2010 (2011 or 2012), the blue bars are the % of the variation between those years.

The length of the red bars all over Figure 20 shows the changes in sales of antimicrobials between the years 2010 (2011 or 2012) and 2016 in a country. The blue bars show how much the sales of antimicrobials have increased or decreased in a given country. Overall the bars (red and blue) show how different countries, have substantially different sales of antimicrobials per year (and kg of animal population or PCU) and how the sales of antimicrobials have changed importantly through the years during which data are available.

9.1.2.1. Addition of the sales of antimicrobials (in mg/kg PCU) during the years 2010 to 2016

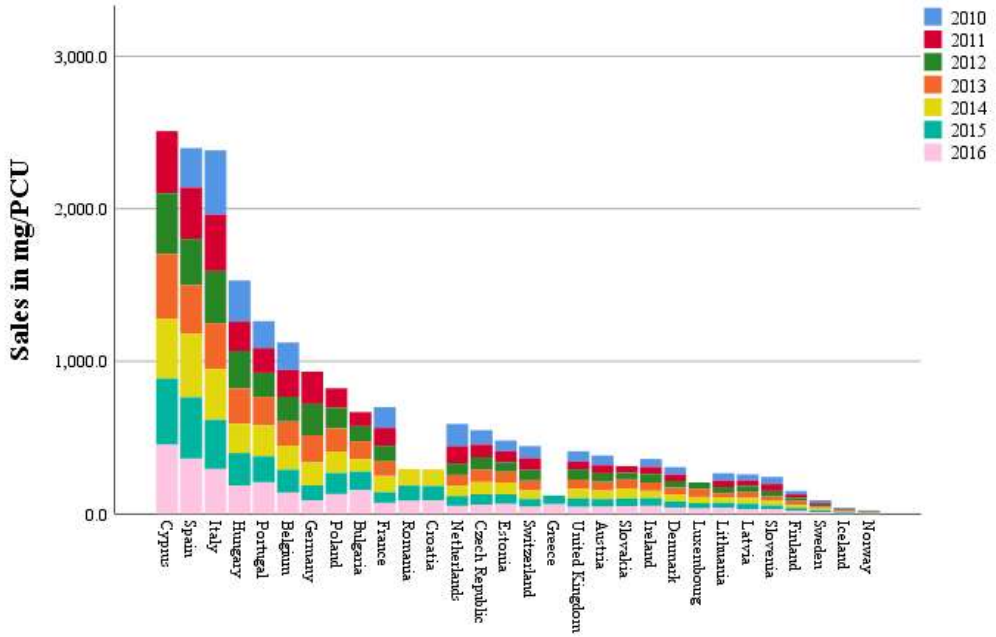


Figure 21. Sales added in mg/kg PCU per country for the years 2010 (2011 or 2012) – 2016, represented as stacked bars. Note: for some of the countries data were not available for some of the years (e.g. Romania, Croatia and Greece) (see Table 4).

Although antibiotics consumed by animals and later on released to the environment might be degraded with time [215], the accumulation of mg/kg PCU of antibiotics over the years 2010 (2011 or 2012) to 2016, shown in Figure 21, raises many questions when considering the presence of antibiotics in the environment, either for its impact on public health [215-218] or because of the accumulation of antimicrobials as a source of contamination into the environment.

It is to be noted that the above figure does not take into account the size of a country, or e.g. livestock density which is something that should be considered if trying to do an analysis of the spatial distribution of antimicrobials into the environment [219]. The addition of the sales of antimicrobials for animal use per mg/kg PCU between the years 2011 and 2016 in Cyprus (surface of 9,251 km^2) is about 2,500 mg/kg PCU. The addition of the sales of antimicrobials for animal use per mg/kg PCU during the same period in Norway (surface of 385,170 km^2) is about 20 mg/kg PCU.

9.1.2.2. Correlations of overall sales of antimicrobials in mg/kg PCU

The Pearson's linear correlation detailed below are significant according to the Bonferroni correction (<0.00147 level, 2-tailed) (see 9.8.).

The overall sales of antimicrobials in mg/kg PCU in 2016 are very strongly correlated with the sales of polymyxins in 2016 (0.861). A similar correlation can be found between the sales of antimicrobials in 2010 and the sales of polymyxins in 2016.

The overall sales in 2016 are strongly correlated with the sales of quinolones in 2012 (0.657). The overall sales in 2010 are strongly correlated with the sales of quinolones during the same period of 2010 (0.664).

The overall sales of antimicrobials in mg/kg PCU in 2016 are very strongly correlated with the sales of premixes in 2016 (0.944). There are also moderate correlations between the sales in 2016 the oral solutions (mg/kg PCU) in 2016, the % of oral solutions, and oral forms in 2016 (see Table 36 for details).

The overall sales (in mg/kg PCU) in 2016 is moderately correlated with the total sales in tonnes in the same year (0.578).

The overall sales in mg/kg PCU in 2010 is strongly correlated with the % of oral forms in 2016 (0.655). The overall sales in 2010 are strongly negatively correlated with the % of individual treatments (-0.655), as well as with the % of injectables in 2016 (-0.650).

The overall sales of antimicrobials in mg/kg PCU in 2016 are strongly correlated with the average temperature (0.768) and with the average high temperature (0.788), and the average low temperature (0.727). Similar correlations are also found with the overall sales in 2010 and the average temperature (0.812) and the average high temperature (0.803).

9.1.3. Results of overall sales of antimicrobials in total tonnes

The sales of antimicrobials in tonnes (without consideration of the animal population) are detailed below.

Table 7. Sales, in tonnes of antimicrobials by country, for the years 2010 to 2016, for 30 European countries[§].

	Sales antimicrobials (tonnes)						
Country	2010	2011	2012	2013	2014	2015	2016
Austria	62.6	53.2	53.0	54.7	53.4	48.5	44.1
Belgium	299.1	297.3	270.5	259.5	265.7	258.1	240.4
Bulgaria	NA	36.9	38.4	46.5	32.6	46.3	61.1
Croatia	NA				31.4	27.9	26.6
Cyprus	NA	51.8	45.0	47.9	41.7	46.9	46.3
Czech Republic	71.2	60.8	53.7	57.3	55.9	47.5	43.2
Denmark	119.0	105.6	107.0	108.5	106.8	101.9	98.7
Estonia	7.6	7.5	7.4	8.5	9.8	8.1	7.2
Finland	11.7	11.4	11.2	11.5	11.4	10.6	9.7

Country	Sales antimicrobials (tonnes)						
	2010	2011	2012	2013	2014	2015	2016
France	999.7	890.3	762.0	681.0	761.5	501.5	513.9
Germany	NA	1818.7	1707.7	1532.1	1305.8	851.1	779.2
Greece	NA					72.6	79.9
Hungary	207.4	147.6	178.7	176.0	150.4	176.0	155.6
Iceland	0.8	0.7	0.7	0.6	0.6	0.6	0.6
Ireland	91.6	82.3	94.8	98.4	88.8	96.4	102.3
Italy	1925.6	1668.3	1534.3	1318.5	1322.0	1300.0	1213.2
Latvia	6.5	6.3	6.7	6.3	6.3	6.8	5.4
Lithuania	16.5	13.9	13.3	9.9	11.9	11.9	12.7
Luxembourg	NA		2.2	2.7	2.1	1.8	1.9
Netherlands	461.1	362.5	245.8	225.6	214.5	213.7	181.7
Norway	6.3	6.2	7.1	6.6	5.8	5.6	5.6
Poland	NA	500.1	528.3	576.6	578.5	582.5	570.2
Portugal	181.5	164.4	156.3	179.4	190.0	169.7	210.9
Romania	NA				272.7	257.1	265.3
Slovakia	NA	10.8	10.2	14.6	16.3	12.6	12.2
Slovenia	8.4	8.4	6.8	4.0	5.7	4.6	5.4
Spain	1804.9	2391.2	2115.6	2202.0	2963.9	3027.8	2724.9
Sweden	12.7	11.3	10.6	10.1	9.3	9.6	9.8
Switzerland	64.5	64.5	56.1	52.2	46.4	41.2	37.6
United Kingdom	455.7	343.9	447.4	422.0	429.6	394.9	321.7

§ NA: Not available

The sales of tablets are excluded from the ESVAC data as those are considered to be mostly for companion animals [61, 62, 64].

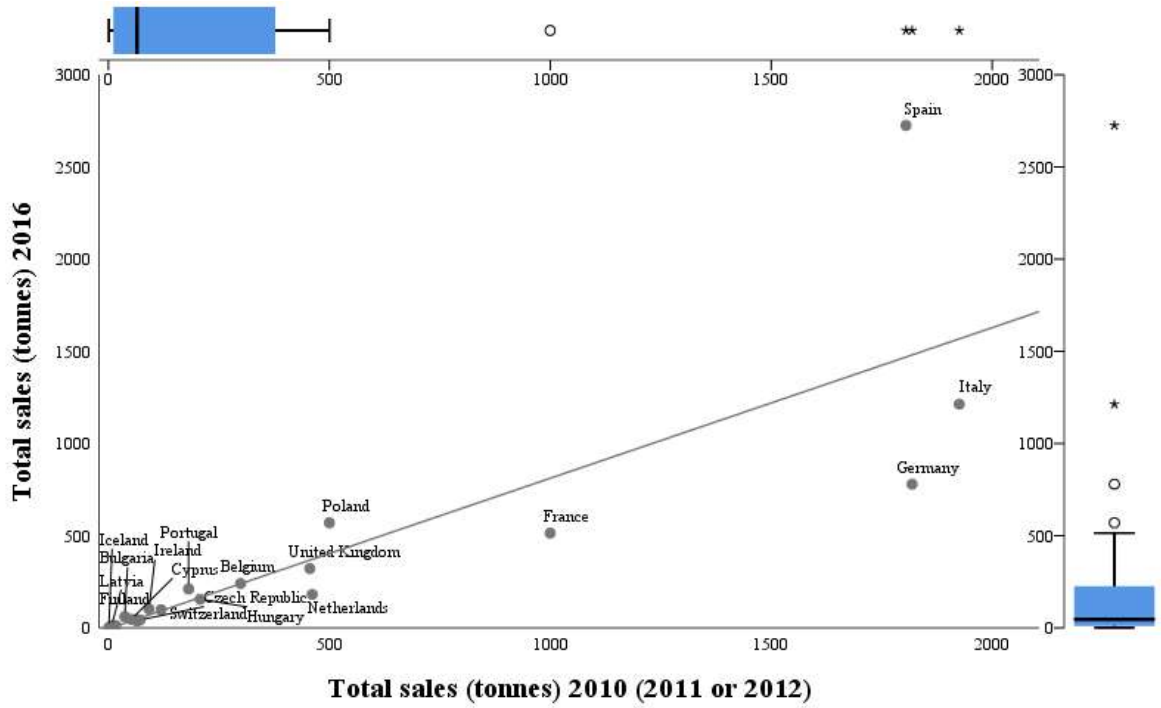


Figure 22. Total tonnes in 2016 versus 2010. For Bulgaria, Cyprus, Germany, Poland and Slovakia the data are from the year 2011, and for Luxembourg, 2012.

It is remarkable how some of the biggest consumers of antimicrobials have substantially reduced their sales in tonnes in the period 2010 (or 2011) to 2016, e.g. Italy (1925.6 to 1213.2), Germany (1818.7 to 779.2) and France (999.7 to 513.9), whilst Spain has increased its sales from 1804.9 to 2724.9 during the same period. In the case of Spain collection of data during the first years was an underestimate.

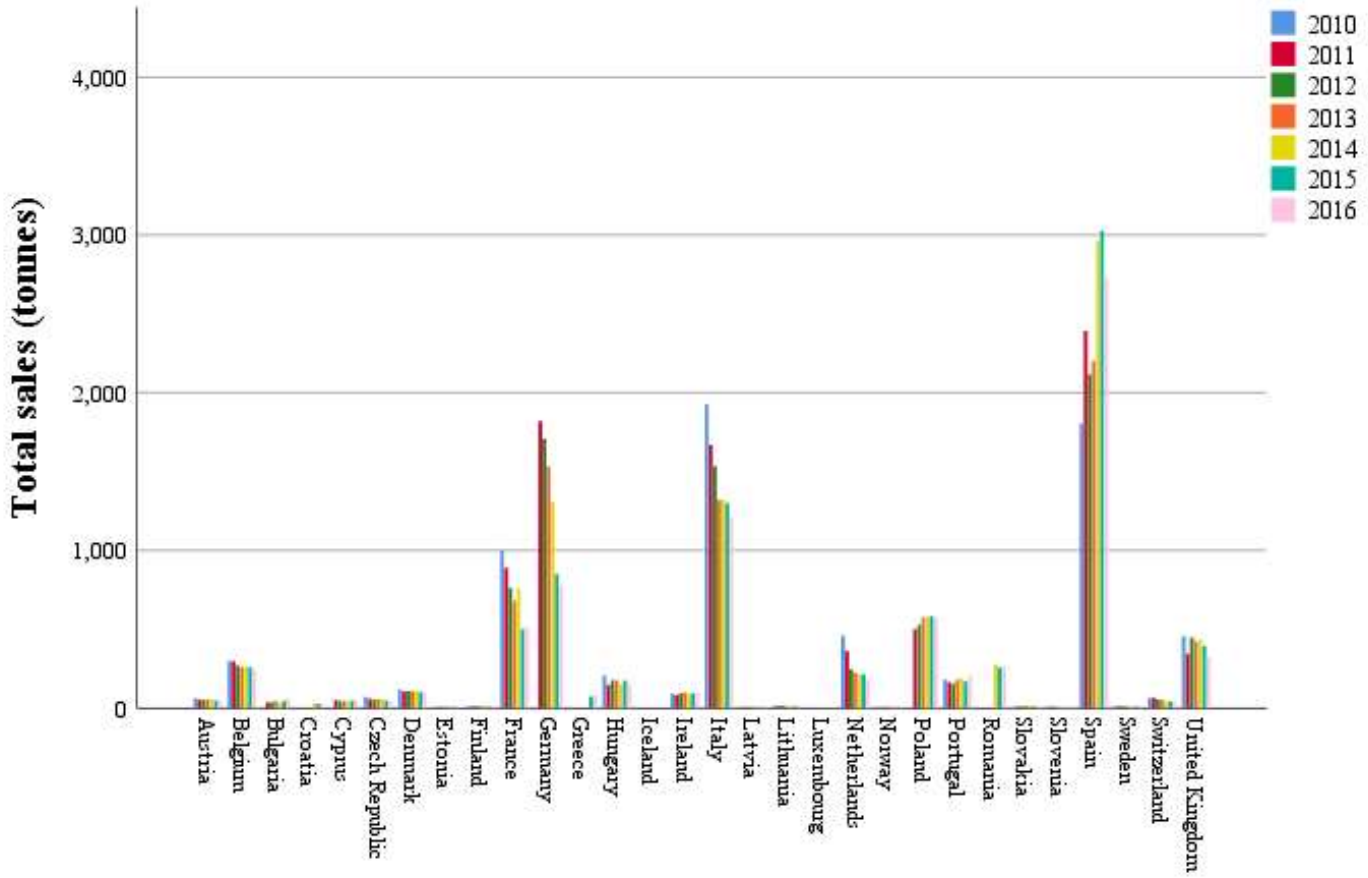


Figure 23. Variations in sales of all antimicrobials between 2010 and 2016 in total tonnes (not taking into account animal production in the country).

The following three figures, Figure 24, Figure 25 and Figure 26 show the total sales (tonnes) per country as three different charts depending on the maximum sales in 2016. The countries represented in each of them and shown in alphabetical order account for: *i)* countries with a lower consumption (up to 40 tonnes), *ii)* countries with middle consumption (40-300 tonnes) and *iii)* countries with higher consumption (more than 300 tonnes).

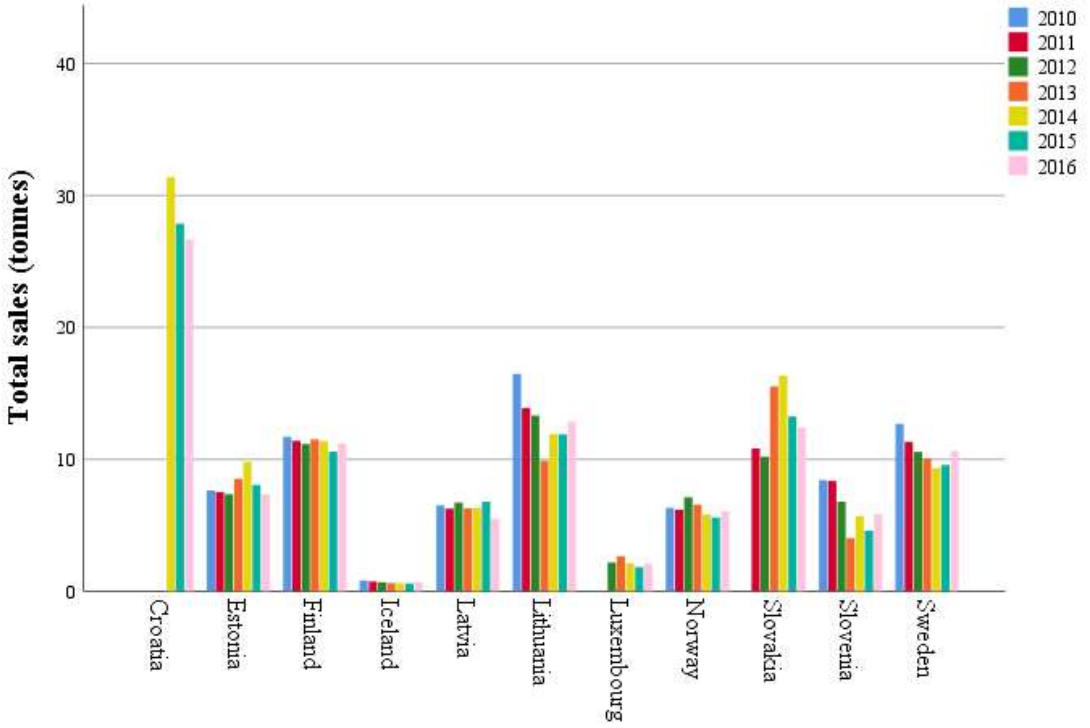


Figure 24. ***Lower consumption countries*** (up to 40 tonnes): Variations in sales of all antimicrobials between 2010 and 2016 in total tonnes (not taking into account animal production in the country), with the countries grouped according to their maximum sales.

In the group of the lower consumption countries, 10 of 11 countries (Croatia, Estonia, Finland, Iceland, Latvia, Lithuania, Luxemburg, Norway, Slovenia and Sweden) have reduced the overall number of tonnes sold. Only Slovakia increased sales.

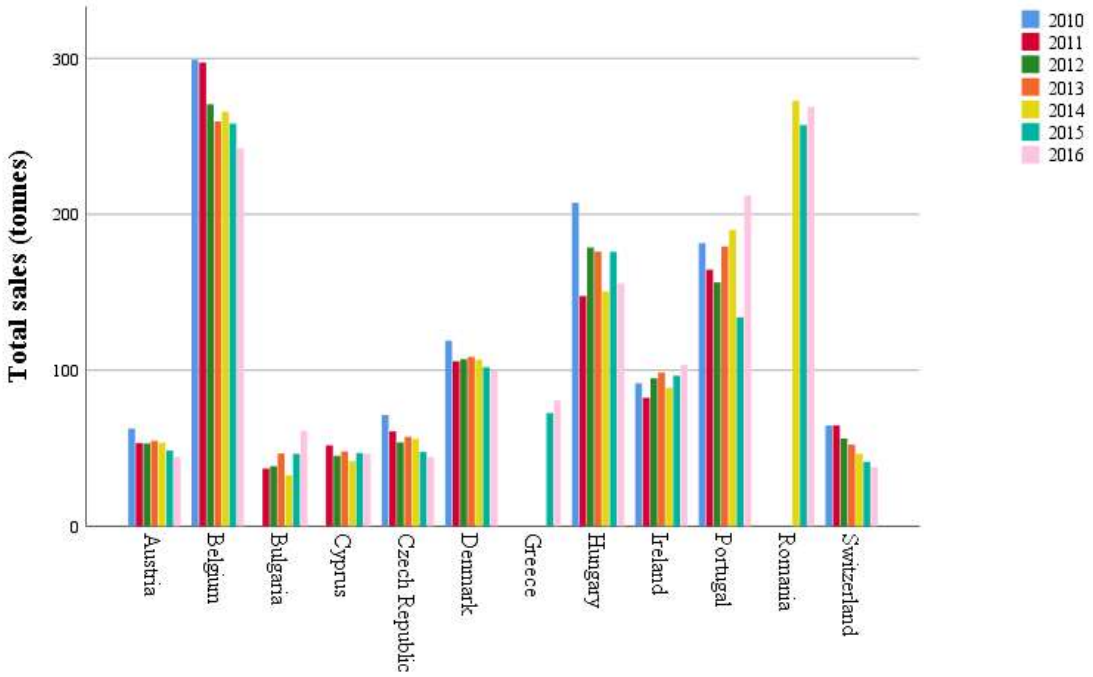


Figure 25. ***Middle consumption countries*** (40 to 300 tonnes): Variations in sales of all antimicrobials between 2010 and 2016 in total tonnes (not taking into account animal production in the country), with the countries grouped according to their maximum sales.

In the group of the middle consumption countries, 8 of 12 countries (Austria, Belgium, Cyprus, Czech Republic, Denmark, Hungary, Romania and Switzerland) have reduced the overall number of tonnes sold. Bulgaria, Greece, Ireland and Portugal increased sales.

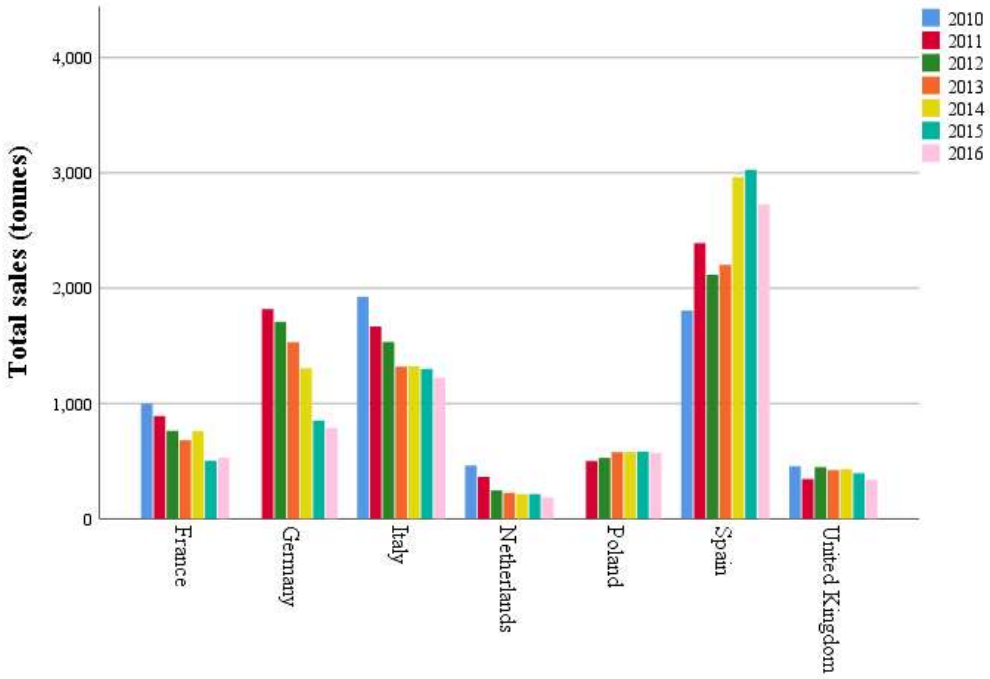


Figure 26. ***Higher consumption countries*** (more than 300 tonnes): Variations in sales of all antimicrobials between 2010 and 2016 in total tonnes (not taking into account animal production in the country), with the countries grouped according to their maximum sales.

In the group of the higher consumption countries, 5 of 7 countries (France, Germany, Italy, Netherlands and United Kingdom) have reduced the overall number of tonnes sold. Poland and Spain increased sales.

Depending on consumption, it can be observed that:

- Countries with a small use of antimicrobials have continued to reduce their use, even if already much smaller than countries with higher use [125, 131].
- Countries like Austria, Belgium and the Czech Republic, in the middle group, have a very active policy on antimicrobial reduction and is evident that this has paid back through the years with an overall reduction of the tonnes consumed [71, 81, 143, 220, 221].
- Whilst sales in tonnes of antimicrobials in some of the biggest countries show a remarkable decrease (e.g. France, Germany and Italy), this is not the case for all of them, most remarkably Spain.

Sales of antimicrobials by weight (tonnes) should be analysed with a proper indicator (e.g. animal production) and adequate context.

Differences in magnitude on scales of Figure 24, Figure 25 and Figure 26 are impressive;

- ***Maximum*** is 31.4 tonnes sold in Croatia (year 2014) in the lower consumption countries and 3,027.8 sold in Spain (year 2015) in the higher consumption countries.
- ***Minimum*** is 0.6 tonnes for Iceland (years 2013-2016), in the lower consumption countries, and 181.7 tonnes sold in the Netherlands (year 2016) in the higher consumption countries.

The aggrupation of countries by selling categories show the different AMC in countries that goes beyond the size and number of animals produced in the country.

Most of the Nordic countries are included in the group of the countries with a lower consumption (with the exception of Denmark that has an exceptionally high pig production). This could be the result of Nordic countries being relatively small countries which results in having a relatively small animal production and consequently an overall small AMC in tonnes but it is also likely to be the result of many years of prudent use of antimicrobials.

Overall it is encouraging to observe how some of the countries with some of the highest sales have continued reducing their sales of antimicrobials in tonnes.

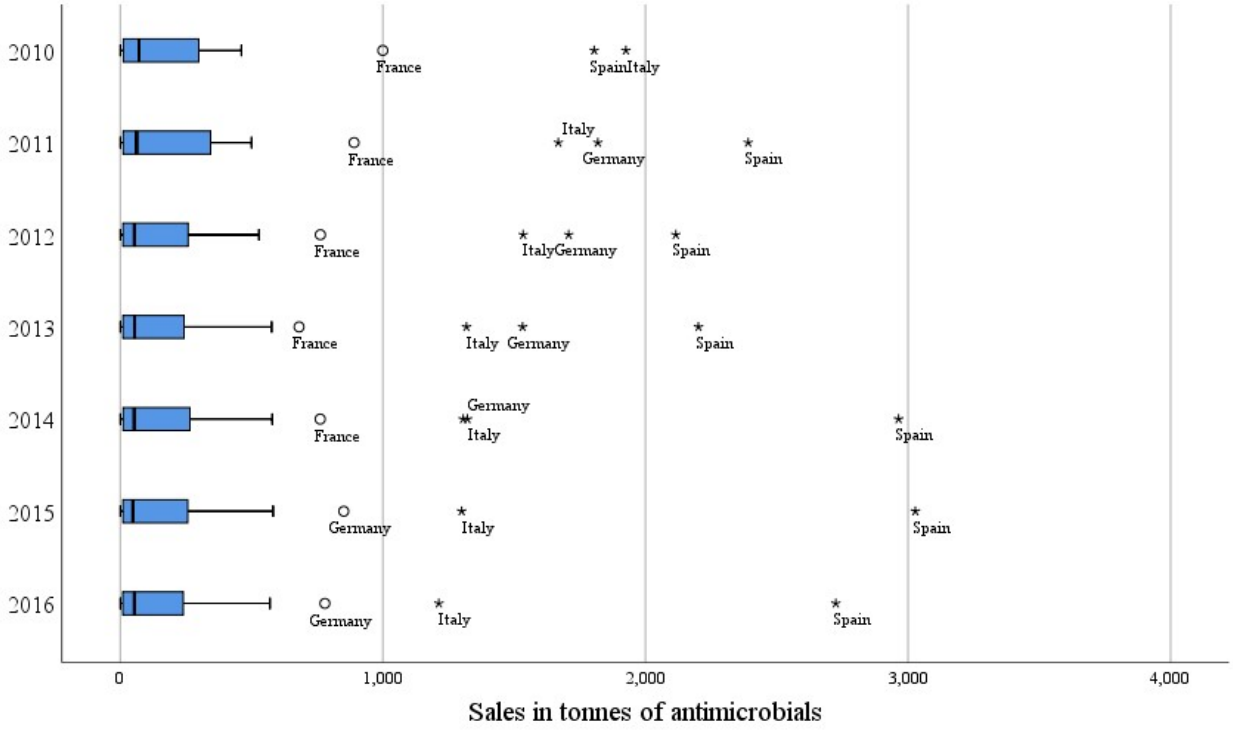


Figure 27. Sales of antimicrobials in tonnes for the years 2010 to 2016. The years in the graph are ordered in increasing order, with data from 2016 at the bottom of the graph.

In all the years, the tonnes of antimicrobials sold in Spain and Italy are more than 3 times the interquartile range considering all countries, Germany was also more than 3 times the interquartile range for the years 2011 to 2013, whilst the value for France exceeds 1.5 times that range in all years except in the last two.

The Pearson's linear correlation of sales of all antimicrobials in mg/kg PCU in 2016 *vs* sales in tonnes of antimicrobials in 2016 is moderate (0.578) and significant with the Bonferroni correction (<0.00147 level, 2-tailed).

9.2. Results of sales of some highly critically important antimicrobials

The sales as mg/kg PCU of some of the HPCIAs are described here below.

9.2.1. Results of sales of 3rd and 4th generation cephalosporins

Table 8. Sales of 3rd and 4th generation cephalosporins in mg/kg PCU, for the years 2010 to 2016 including the variation in the percentage of sales between the years 2010 and 2016*.

Country	3rd and 4th generation cephalosporins							Percentage variation in 3rd and 4th generation cephalosporins mg/kg PCU, years 2010 to 2016*
	2010	2011	2012	2013	2014	2015	2016	
Austria	0.30	0.33	0.33	0.35	0.18	0.21	0.22	-26.7%
Belgium	***0.51***	0.50	0.49	0.50	0.47	0.43	0.30	-41.2%
Bulgaria	NA	0.05	0.03	0.12	0.06	0.20	0.10	100.0%
Croatia	NA				0.13	0.20	0.16	Not computed
Cyprus	NA	0.17	0.46	0.49	***0.79***	0.35	0.70	***311.8%***
Czech Republic	0.37	0.28	0.34	0.41	0.40	0.41	0.41	10.8%
Denmark	0.05	0.03	0.03	0.02	0.02	0.01	0.01	-80.0%
Estonia	0.36	0.55	0.61	0.66	0.63	0.61	***0.73***	102.8%
Finland	0.00 (0.009)	0.02	0.03	0.02	0.02	0.01	0.00 (0.006)	-33.3%
France	0.31	0.30	0.31	0.29	0.28	0.21	0.06	-80.6%
Germany	NA	0.40	0.44	0.43	0.42	0.41	0.38	-5.0%
Greece	NA					0.09	0.10	Not computed
Hungary	0.27	0.14	0.32	0.31	0.25	0.38	0.42	55.6%
Iceland	0.01	0.01	***0.00***	***0.00***	0.01	***0.00***	***0.00 (0.0026)***	***-126.0%***
Ireland	0.06	0.07	0.12	0.10	0.13	0.11	0.13	116.7%
Italy	0.35	0.36	0.40	0.38	0.41	0.40	0.38	8.6%

Country	3rd and 4th generation cephalosporins							Percentage variation in 3rd and 4th generation cephalosporins mg/kg PCU, years 2010 to 2016*
	2010	2011	2012	2013	2014	2015	2016	
Latvia	0.22	0.23	0.40	0.40	0.37	0.36	0.26	18.2%
Lithuania	NA	0.04	0.05	0.17	0.18	0.05	0.13	225.0%
Luxembourg	NA		***0.68***	***0.68***	0.63	***0.62***	0.73	7.4%
Netherlands	NA	0.19	0.02	0.01	***0.00***	***0.00***	***0.00 (0.0005)***	-102.5%
Norway	***0.00 (0.0007)***	***0.00***	***0.00***	***0.00***	***0.00***	***0.00***	***0.00 (0.0003)***	-57.1%
Poland	NA	0.09	0.13	0.17	0.17	0.14	0.16	77.8%
Portugal	0.30	0.32	0.24	0.37	0.43	0.46	0.46	53.3%
Romania	NA				0.05	0.04	0.08	Not computed
Slovakia	NA	***0.65***	0.53	0.40	0.46	0.35	0.36	-44.6%
Slovenia	0.11	0.09	0.17	0.12	0.14	0.17	0.16	45.5%
Spain	NA	0.26	0.26	0.28	0.33	0.31	0.30	15.4%
Sweden	0.02	0.02	0.01	0.01	***0.00***	***0.00***	***0.00 (0.003)***	-115.0%
Switzerland	0.24	0.24	0.25	0.25	0.22	0.20	0.16	-33.3%
United Kingdom	0.21	0.17	0.20	0.18	0.19	0.17	0.14	-33.3%

*For some countries (Croatia, Greece and Romania) data were not collected until after the year 2013, and the % of change was not computed.

§ NA: Not available

Blue, bold and italics highlight the lower result or % value per column. Red, bold and italics the highest result or % per column.

When there are sales of 3rd and 4th generation of cephalosporins, however small they are, and therefore they are not zero, detailed more decimals are specified and taken into account for the calculation of the % of variation.

For Bulgaria, Cyprus, Germany, the Netherlands, Poland and Slovakia the data are from the year 2011, and for Luxembourg, from 2012.

As indicated in the latest ESVAC reports [65, 71], sales of 3rd and 4th cephalosporins in the countries where most of its use is for companion animals can represent a considerable overestimate of those sales.

The lowest sales of 3rd and 4th cephalosporins per mg/kg PCU were for Norway (all years), Iceland (years 2012, 2013, 2015 and 2016), the Netherlands (2014, 2015 and 2016) and Sweden (2014, 2015 and 2016). The sales in all those countries of 3rd and 4th cephalosporins are expressed on the ESVAC reports as below 0.01 mg/kg PCU, but more detailed figures can be obtained from the ESVAC online database once the data are downloaded [188].

The highest sales of 3rd and 4th generation cephalosporins in mg/kg PCU were for Belgium (0.51 mg/kg PCU year 2010), Slovakia (0.65 mg/kg PCU year 2011), Luxembourg (0.68 mg/kg PCU years 2012 and 2013, 0.62 mg/kg PCU year 2015), Cyprus (0.79 mg/kg PCU year 2014) and Estonia (0.73 mg/kg PCU year 2016).

The biggest decrease in sales of 3rd and 4th cephalosporins sold per mg/kg PCU was for Iceland (-126.0%, years 2010 to 2016) and the highest increase for Cyprus (311.8%, years 2011 to 2016).

Table 9. Overall sales statistics of 3rd and 4th generation cephalosporins in mg/kg PCU in years 2010 to 2016.

		3rd and 4th generation cephalosporins (mg/kg PCU)						
		2010	2011	2012	2013	2014	2015	2016
Valid countries		18	26	27	27	29	30	30
Missing countries*		12	4	3	3	1	0	0
Mean		0.21	0.21	0.25	0.26	0.25	0.23	0.24
Std. Deviation		0.15	0.18	0.20	0.20	0.21	0.18	0.22
Minimum		0.00	0.00	0.00	0.00	0.00	0.00	0.00
Maximum		0.51	0.65	0.68	0.68	0.79	0.62	0.73
Percentiles	25	0.04	0.05	0.03	0.10	0.06	0.05	0.08
	50	0.23	0.18	0.25	0.28	0.19	0.21	0.16
	75	0.32	0.32	0.40	0.40	0.41	0.39	0.38

*Countries are not included by year:
2010: Bulgaria, Croatia, Cyprus, Germany, Greece, Lithuania, Luxembourg, Netherlands, Poland, Romania, Slovakia and Spain.
2011: Croatia, Greece, Luxembourg and Romania.
2012: Croatia, Greece and Romania.
2013: Croatia, Greece and Romania.
2014: Greece.

The variation in the % of 3rd and 4th generation cephalosporins expressed as mg/kg PCU of the years 2010 to 2016 has a range between an increase of 331.8% of cephalosporins and a decrease of -126.0%. Although the average shows a 13.7% increase in the use of these cephalosporins, it should be noted that the range of variation is very high (std = 99.0%).

The % variation of 3rd and 4th generation cephalosporins expressed as mg/kg PCU between 2010 (or 2011, 2012) (0.23) and 2016 (0.24), by one-sample t-test, is not statistically different from zero ($p = 0.478$, 2-tailed).

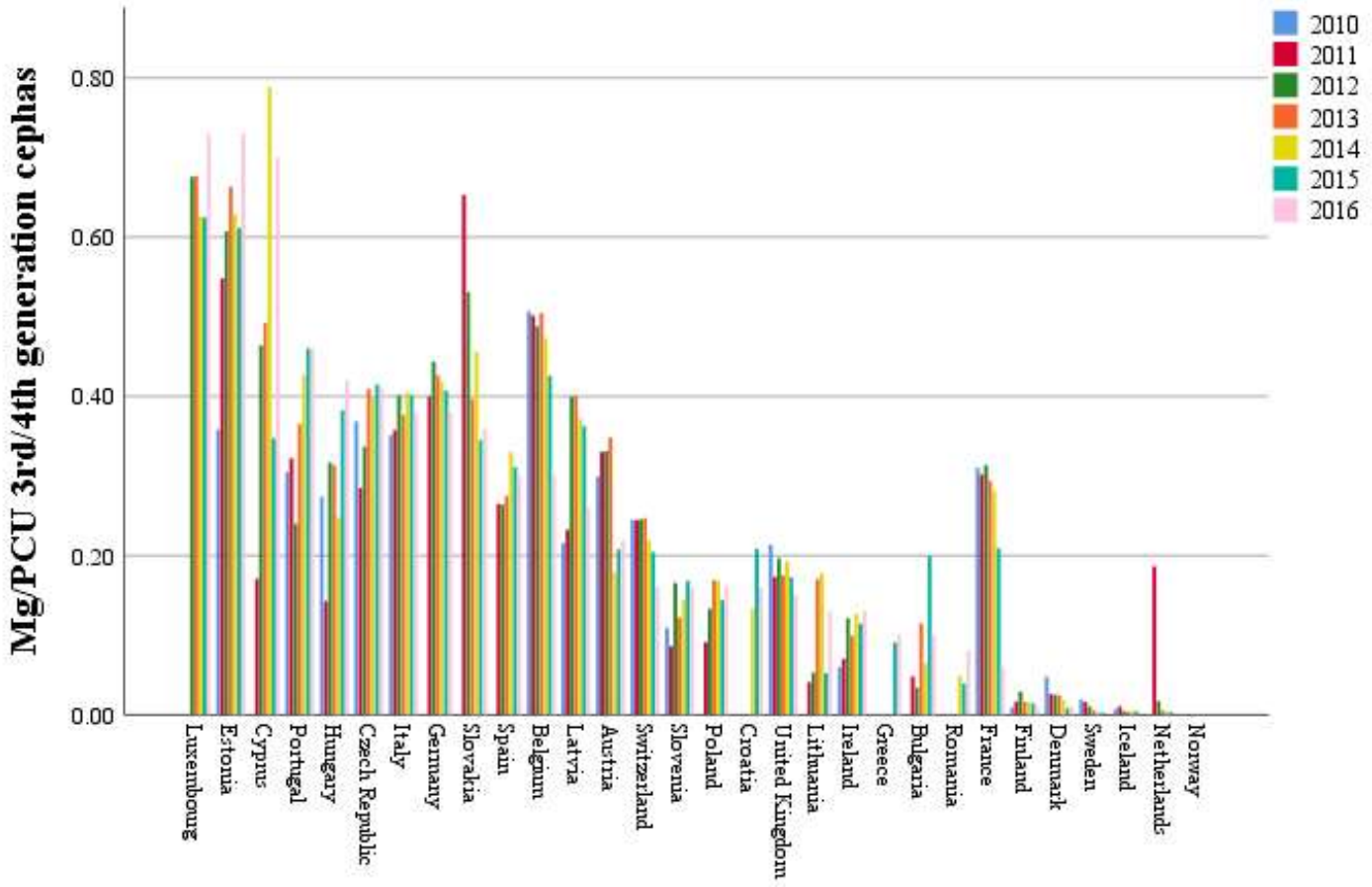


Figure 28. Overall sales of 3rd and 4th generation cephalosporins in mg/kg PCU for all years (2010 to 2016) for all countries reporting data to ESVAC, ordered by sales (decreasing) in the year 2016.

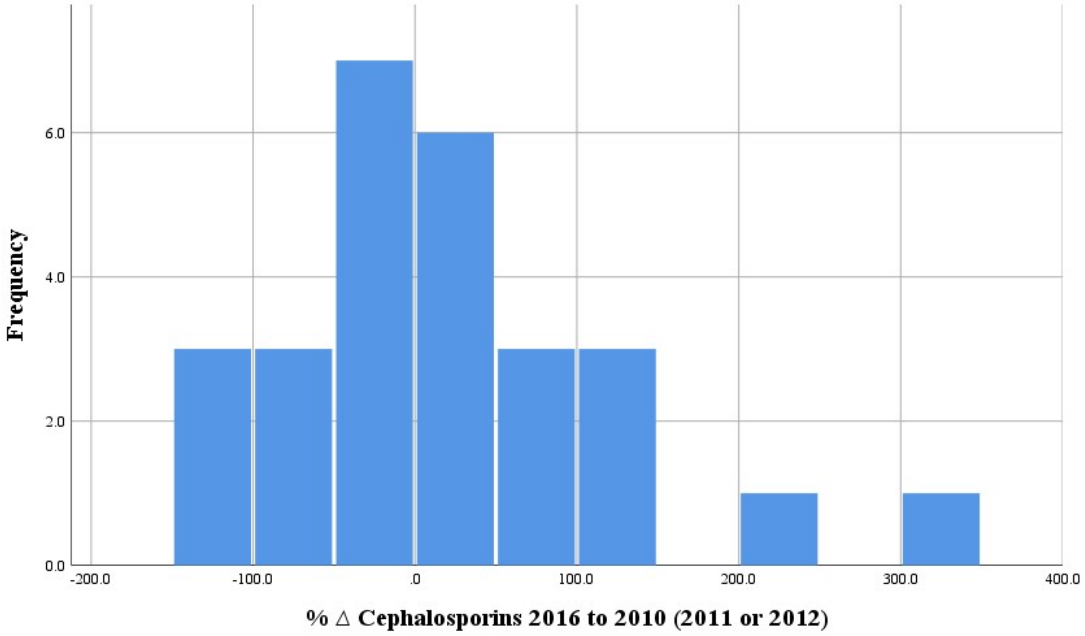


Figure 29. Variation in decrease and increase in the percentage of 3rd and 4th generation cephalosporins between 2016 and 2010 (or 2011, 2012).

Thirteen countries decreased the sales of 3rd and 4th generation cephalosporins between 2016 and 2010 (2010 or 2012), whilst 14 countries increased such sales.

Considering that 3rd and 4th generation cephalosporins are HPCIA according to the WHO [8, 155, 157-160], the values shown in Figure 29 are worrisome.

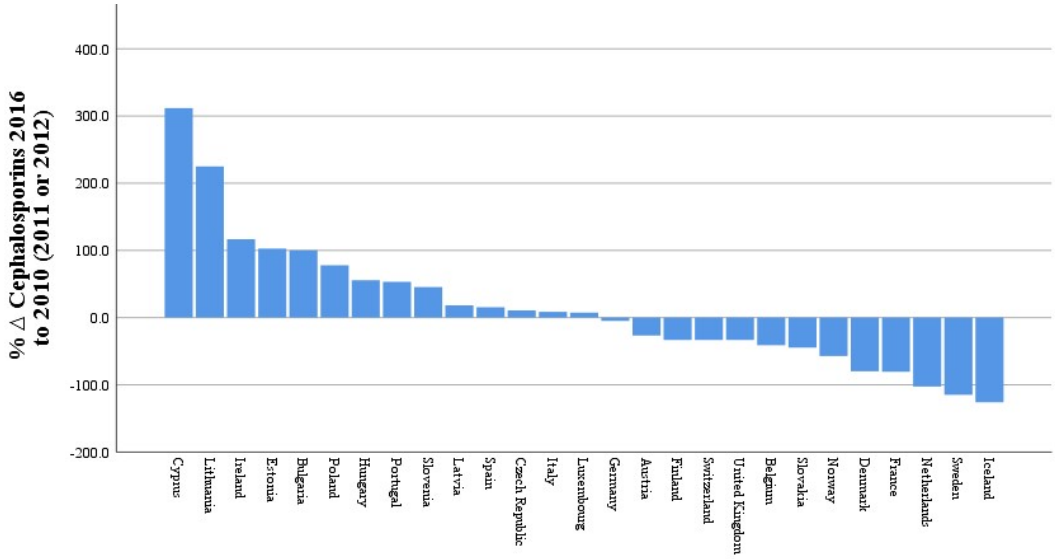


Figure 30. Variation in the percentage of sales of 3rd and 4th generation cephalosporins between the years 2010 (2011 or 2012 as appropriate) and 2016 (27 countries) by decreasing the percentage of sales.

The following countries have decreased their sales of 3rd and 4th generation cephalosporins between the years 2010 (2011 or 2012) and 2016: Iceland (-126%), Sweden (-115%), Netherlands (-102.5%), France (-80.6%), Denmark (-80%), Norway (-57.1%), Slovakia (-44.6%), Belgium (-41.2%), Finland (-33.3%), Switzerland (-33.3%), United Kingdom (-33.3%), Austria (-26.7%) and Germany (-5%).

Whilst others increased the sales during the same period: Cyprus (311.8%), Lithuania (225%), Ireland (116.7%), Estonia (102.8%), Bulgaria (100%), Poland (77.8%), Hungary (55.6%), Portugal (53.3%), Slovenia (45.5%), Latvia (18.2%), Spain (15.4%), Czech Republic (10.8%), Italy (8.6%) and Luxembourg (7.4%).

As above, the number of countries that have importantly increased the mg/kg PCU of 3rd and 4th generation cephalosporins through the years is of concern. On the other hand, the number of countries that have decreased such use is encouraging.

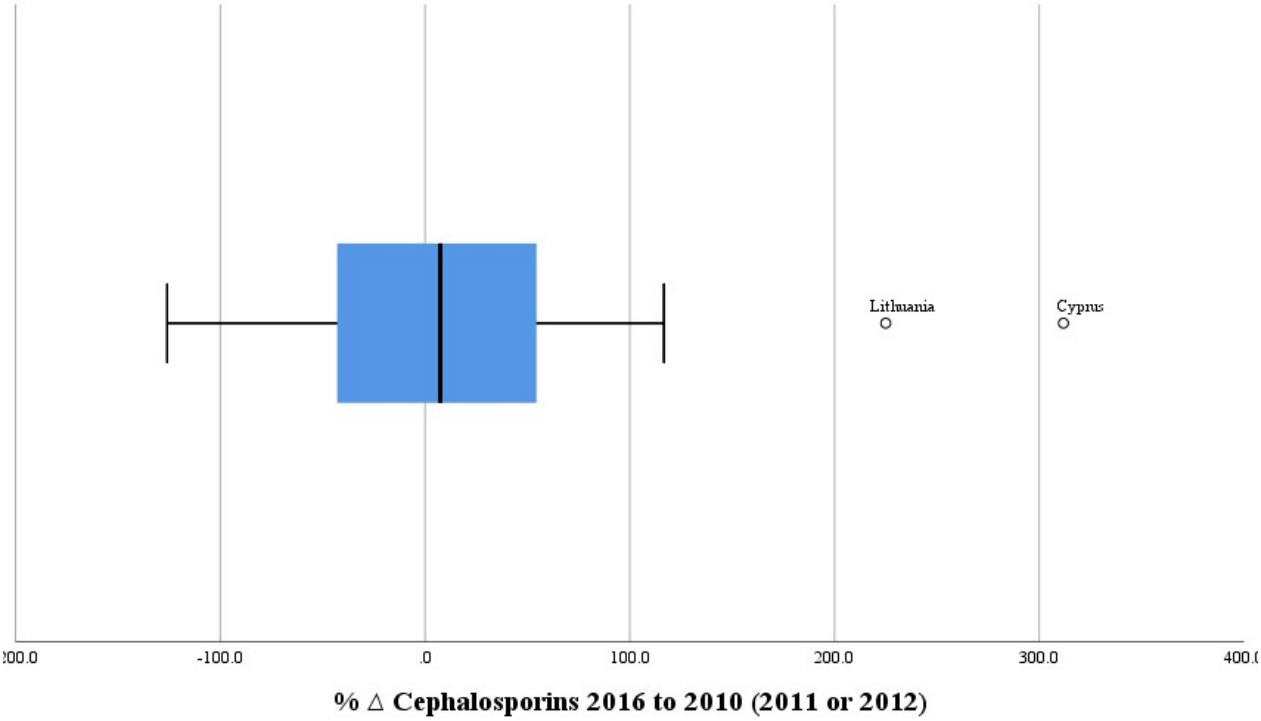


Figure 31. Box plot of variation in the percentage of 3rd and 4th generation cephalosporins between 2010 and 2016.

The resulting mean is a not statistically different from zero increase in sales of 3rd and 4th generation cephalosporins of 13.7%, which is not as it could be expected of such an important class of antimicrobials, where a decrease in sales would be desirable.

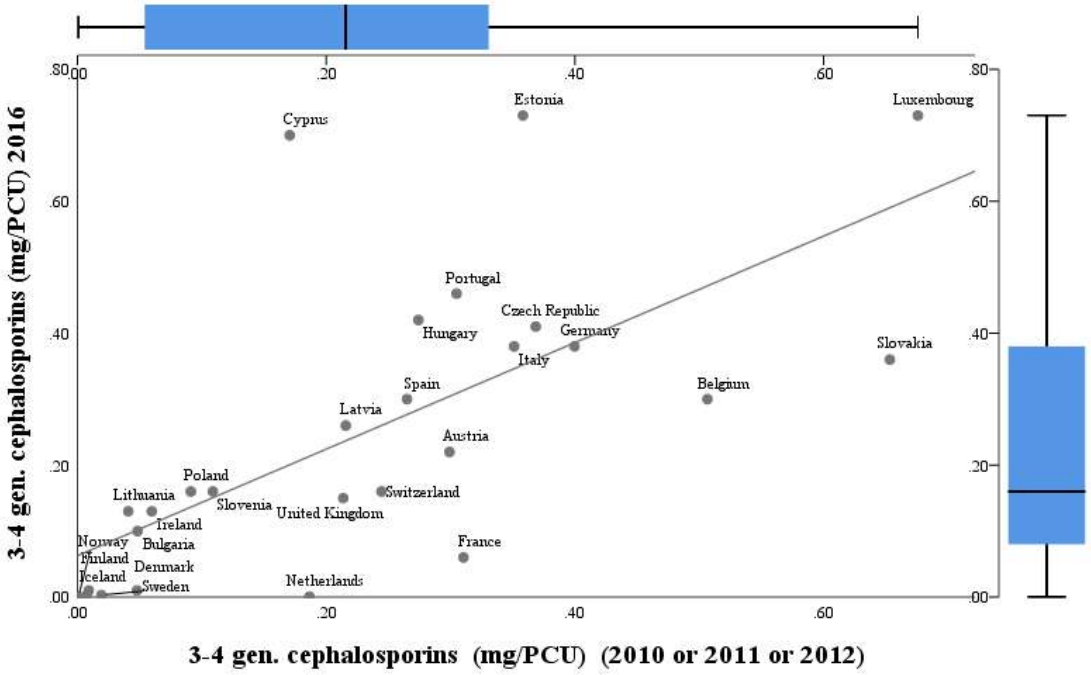


Figure 32. 3rd and 4th generation cephalosporins in mg/kg PCU in 2016 *vs* 2010 (2011 or 2012).

The Pearson's linear correlations of the sales of 3rd and 4th generation cephalosporins in 2016 with the sales of 3rd and 4th generation cephalosporins in 2010 is strong (0.679) and significant, within the Bonferroni correction (<0.00147 level, 2-tailed).

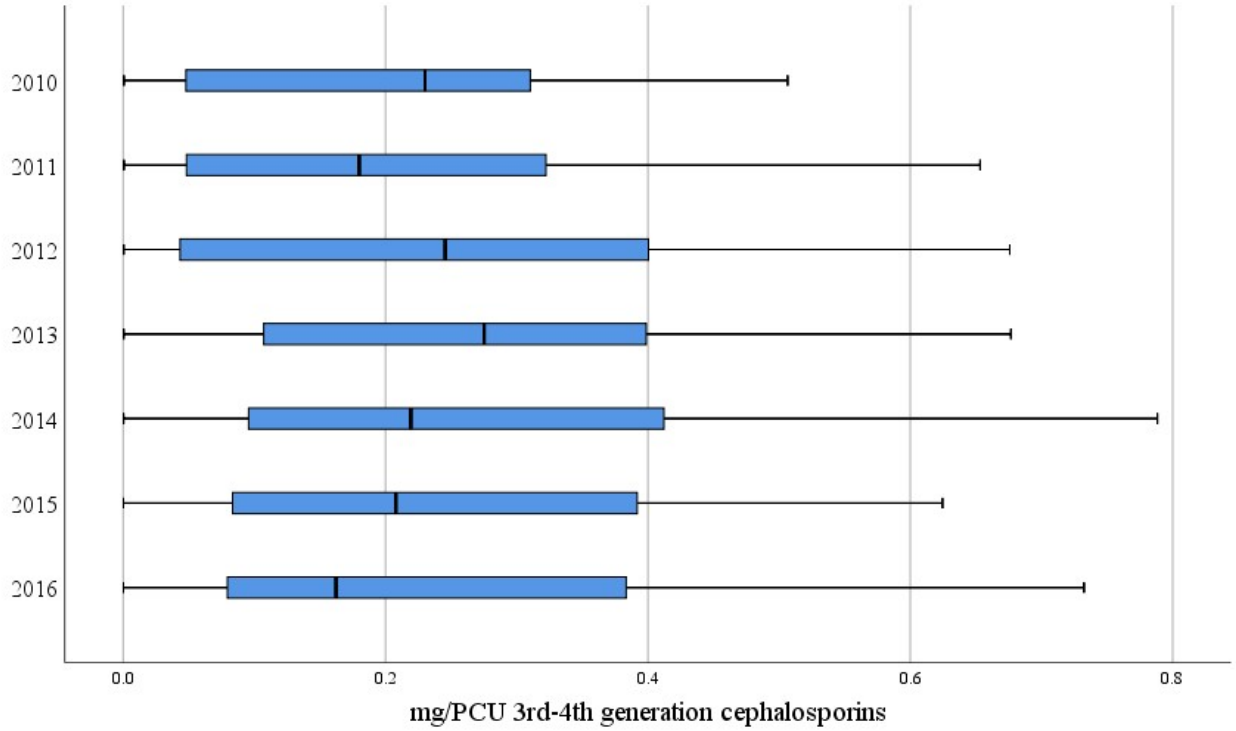


Figure 33. Variations in sales of 3rd and 4th generation cephalosporins between 2010 (or 2011, 2012) and 2016 in mg/kg PCU. The years in the graph are ordered in increasing order, with data from 2016 at the bottom of the graph.

9.2.1.1. Correlation of sales of 3rd and 4th generation cephalosporins in 2015 with the overall sales of antimicrobials in the country in 2016

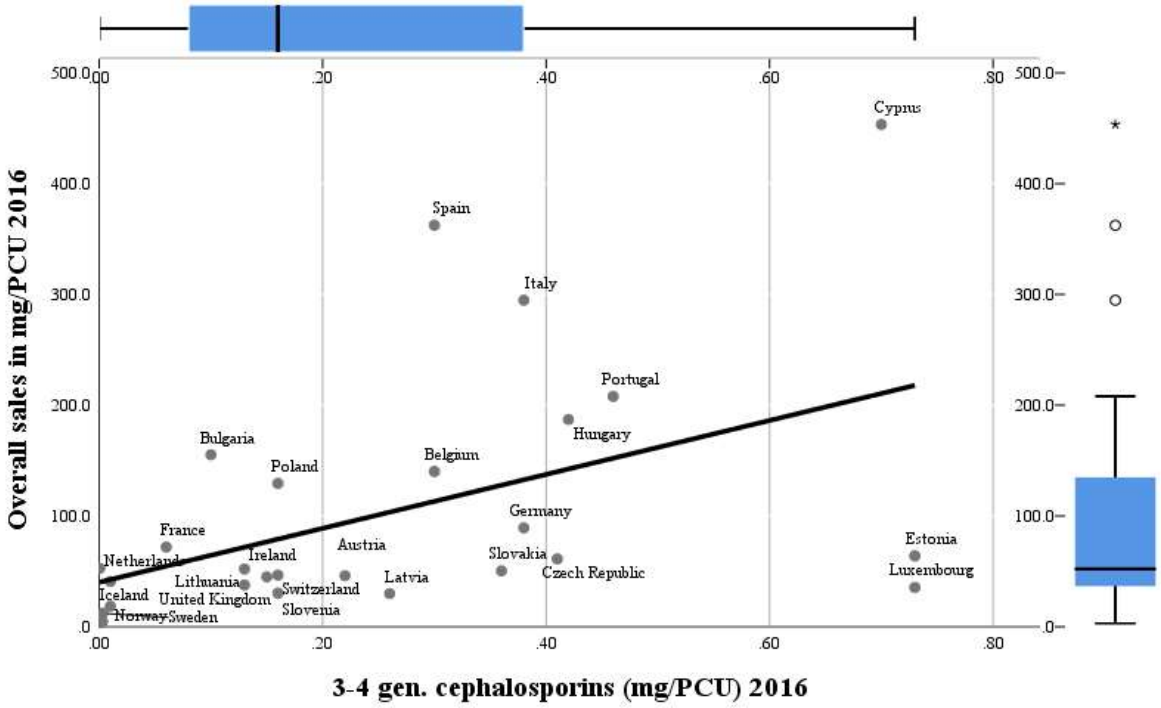


Figure 34. Overall sales of antimicrobials *vs* sales of 3rd and 4th generation cephalosporins in 2016.

The high use of 3rd and 4th generation cephalosporins, and total antimicrobials, in Cyprus combined with a high overall antimicrobial sale is noticeable.

The high use of cephalosporins with lower overall use of antimicrobial for Estonia and Luxembourg is remarkable. They also stand out, but for the low values, the total sales of cephalosporins of 3rd and 4th generation (zero or almost zero), in mg/kg PCU, in Denmark, Finland, Iceland, the Netherlands, Norway and Sweden, with low values of sales.

The Pearson's linear correlation of the overall sales of antimicrobials *vs* sales of 3rd and 4th generation cephalosporins in 2016 is moderate (0.485) and significant at the 0.01 level (2-tailed), but not at the cut-off point of the Bonferroni correction (see 9.8.).

9.2.1.2. Other relevant correlations of 3rd and 4th generation cephalosporins

Of the analysis of all the Pearson's linear correlations of 3rd and 4th generation cephalosporins with other indicators, the only correlation which is significant at the cut-off point of the Bonferroni correction is the above detailed strong correlation (0.679) between the sales of 3rd and 4th generation cephalosporins in 2016 with the sales of 3rd and 4th generation cephalosporins in 2010, this is not the case of the correlations with quinolones and polymyxins, as described further below.

9.2.2. Results of sales of quinolones

The analysis of the group quinolones is composed of what at the ESVAC reports are presented as fluoroquinolones plus other quinolones, i.e. comprises all the substances classified as quinolones.

Table 10. Sales of quinolones in mg/kg PCU, for the years 2010 to 2016 including the variation in the percentage of sales between the years 2010 and 2016*.

Country	Quinolones							Percentage variation in quinolones mg/kg PCU, years 2010 to 2016
	2010	2011	2012	2013	2014	2015	2016	
Austria	0.60	0.59	0.52	0.59	0.49	0.53	0.51	-15.0%
Belgium	2.35	2.37	2.50	1.96	2.04	2.32	0.94	-60.0%
Bulgaria	NA	5.39	6.48	6.93	1.79	5.65	5.23	-3.0%
Croatia	NA				4.20	3.99	3.10	Not computed
Cyprus	NA	2.03	3.65	1.17	1.53	1.50	2.12	4.4%
Czech Republic	1.53	1.67	1.88	1.80	1.78	1.73	1.70	11.1%
Denmark	0.34	0.15	0.86	0.40	0.71	0.42	0.37	8.8%
Estonia	2.67	2.32	1.08	1.65	1.56	1.80	1.29	-51.7%
Finland	0.15	0.16	0.16	0.16	0.18	0.14	0.15	0%
France	1.75	1.45	1.34	1.27	1.43	0.74	0.67	-61.7%
Germany	NA	0.91	1.20	1.38	1.37	1.14	1.02	12.1%
Greece	NA					4.32	6.98	Not computed
Hungary	9.07	6.94	***11.20***	9.39	9.40	9.71	***9.79***	7.9%
Iceland	0.24	0.34	0.15	***0.04***	***0.00***	***0.00***	***0.01***	***-95.8%***
Ireland	0.38	0.40	0.57	0.50	0.36	0.41	0.48	26.3%
Italy	***12.37***	***11.30***	9.25	7.20	7.06	6.18	4.75	-61.6%
Latvia	4.12	2.22	1.72	2.14	1.60	1.11	0.85	-79.4%
Lithuania	NA	0.60	0.81	1.26	4.00	1.91	1.05	***75.0%***

Country	Quinolones							Percentage variation in quinolones mg/kg PCU, years 2010 to 2016
	2010	2011	2012	2013	2014	2015	2016	
Luxembourg	NA		0.68	1.52	0.75	0.88	0.83	22.1%
Netherlands	NA	1.60	0.93	0.88	1.20	1.26	0.98	-38.8%
Norway	0.21	0.13	0.75	0.38	0.06	0.05	0.04	-81.0%
Poland	NA	7.28	8.35	8.90	9.11	8.58	9.69	33.1%
Portugal	6.25	8.85	9.41	8.44	***11.55***	8.92	9.01	44.2%
Romania	NA				5.49	6.34	3.48	Not computed
Slovakia	NA	3.32	3.25	2.95	4.23	2.95	3.66	10.2%
Slovenia	2.70	6.02	4.10	1.77	3.97	3.06	2.95	9.3%
Spain	NA	9.80	10.87	***9.89***	10.84	***9.74***	9.29	-5.2%
Sweden	***0.13***	***0.10***	***0.10***	0.05	0.03	0.02	0.07	-46.2%
Switzerland	NA				0.47	0.47	0.35	Not computed
United Kingdom	0.30	0.28	0.33	0.36	0.35	0.35	0.23	-23.3%

*For some countries (Croatia, Greece and Romania) data were not collected until after the year 2013, and the percentage of change was not computed.
For Switzerland, the data for "other quinolones" were not available until the year 2014 for confidentiality reasons.
For Bulgaria, Cyprus, Germany, the Netherlands, Poland and Slovakia the data are from the year 2011, and for Luxembourg, 2012.
§ NA: Not available
Blue, bold and italics highlight the lower result or percentage value per column. Red, bold and italics the highest.

The lowest sales of quinolones per mg/kg PCU were for Sweden (0.13, 0.10 and 0.10 mg/kg/PCU for the years 2010 to 2012 respectively) and Iceland (0.04, 0.00, 0.00 and 0.01 mg/kg PCU for the years 2013 to 2016 respectively).

The highest sales of quinolones in mg/kg PCU were for Italy (12.37 and 11.30 mg/kg PCU for the years 2010 and 2011 respectively), Hungary (11.20 and 9.79 mg/kg PCU for the years 2012 and 2016 respectively), Spain (9.89 and 9.74 mg/kg PCU for the years 2013 and 2015 respectively) and Portugal (11.55 mg/kg PCU for the year 2014).

The biggest decrease of sales of quinolones sold per mg/kg PCU was for Iceland (-95.8%, years 2010 to 2016) and the highest increase for Lithuania (75.0%, years 2011 to 2016).

Table 11. Overall sales statistics of all quinolones in mg/kg PCU in the years 2010 to 2016.

		Quinolones (mg/kg PCU)						
		2010	2011	2012	2013	2014	2015	2016
Valid countries		17	25	26	26	29	30	30
Missing countries*		13	5	4	4	1	0	0
Mean		2.66	3.05	3.16	2.81	3.02	2.87	2.72
Std. Deviation		3.51	3.39	3.64	3.28	3.43	3.12	3.18
Minimum		0.13	0.10	0.10	0.04	0.00	0.00	0.01
Maximum		12.37	11.30	11.20	9.89	11.55	9.74	9.79
Percentiles	25	0.27	0.37	0.65	0.48	0.48	0.46	0.45
	50	1.53	1.67	1.27	1.45	1.56	1.62	1.04
	75	3.41	5.71	4.76	3.95	4.22	4.65	3.93

*Countries are not included by year:
2010: Bulgaria, Croatia, Cyprus, Germany, Greece, Lithuania, Luxembourg, Netherlands, Poland, Romania, Slovakia, Spain and Switzerland.
2011: Croatia, Greece, Luxembourg, Romania and Switzerland.
2012: Croatia, Greece, Romania and Switzerland.
2013: Croatia, Greece and Romania and Switzerland.
2014: Greece.

The variation in the percentage of quinolones expressed as mg/kg PCU of the years 2010 to 2016 of 26 countries providing data to ESVAC has a range between -95.8 and +75.0 percent of the variation, with the mean being a decrease of -13.8%, and a standard deviation of 42.8%.

The percentage variation of sales of quinolones expressed as mg/kg PCU between 2010 (or 2011, 2012) (2.95) to 2016 (2.72), by one-sample t-test, is not statistically different from zero ($p = 0.113$, 2-tailed).

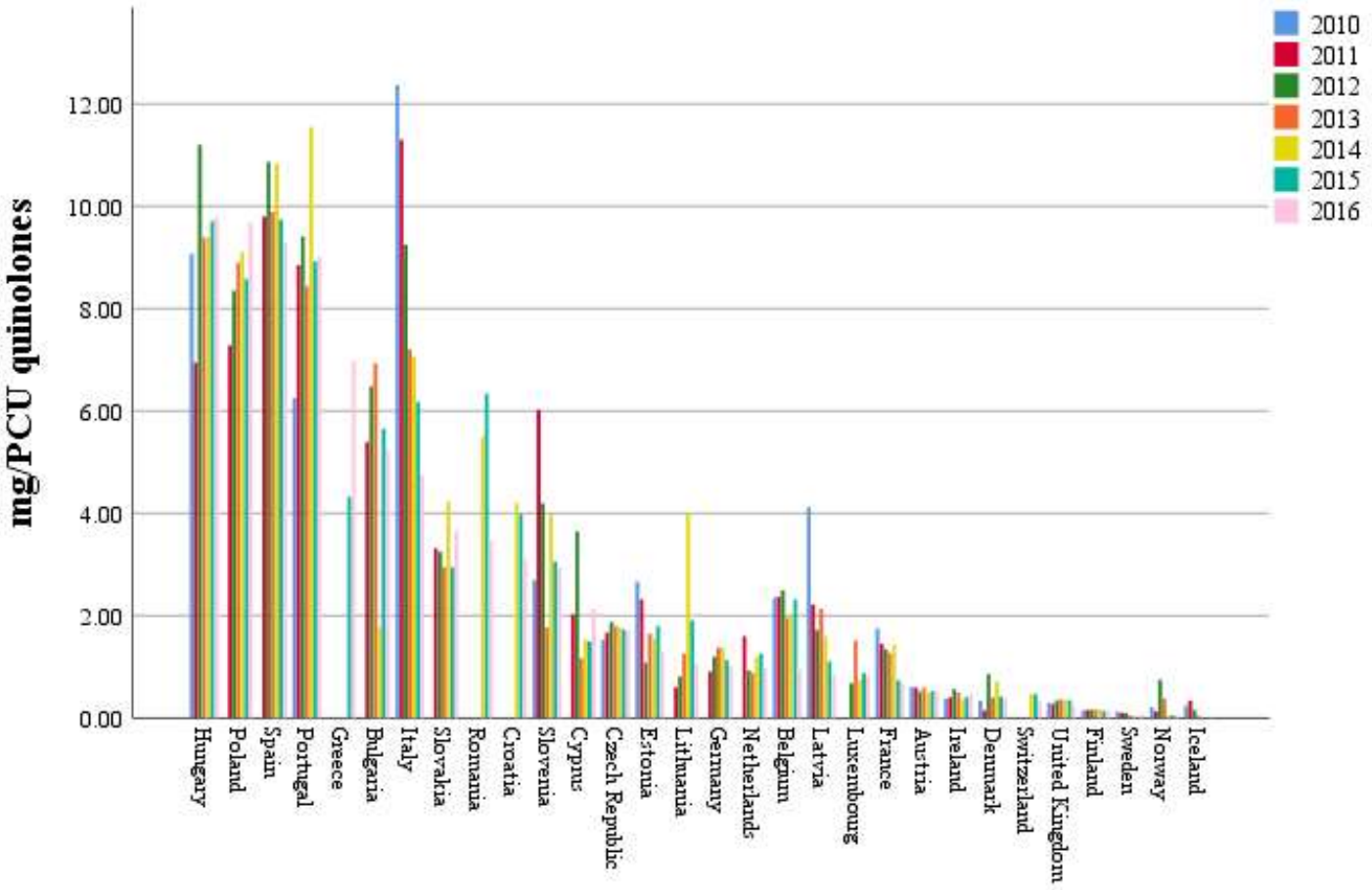


Figure 35. Overall sales of all quinolones in mg/kg PCU for all years (2010 to 2016) for all countries reporting data to ESVAC, ordered by sales (decreasing) in the year 2016.

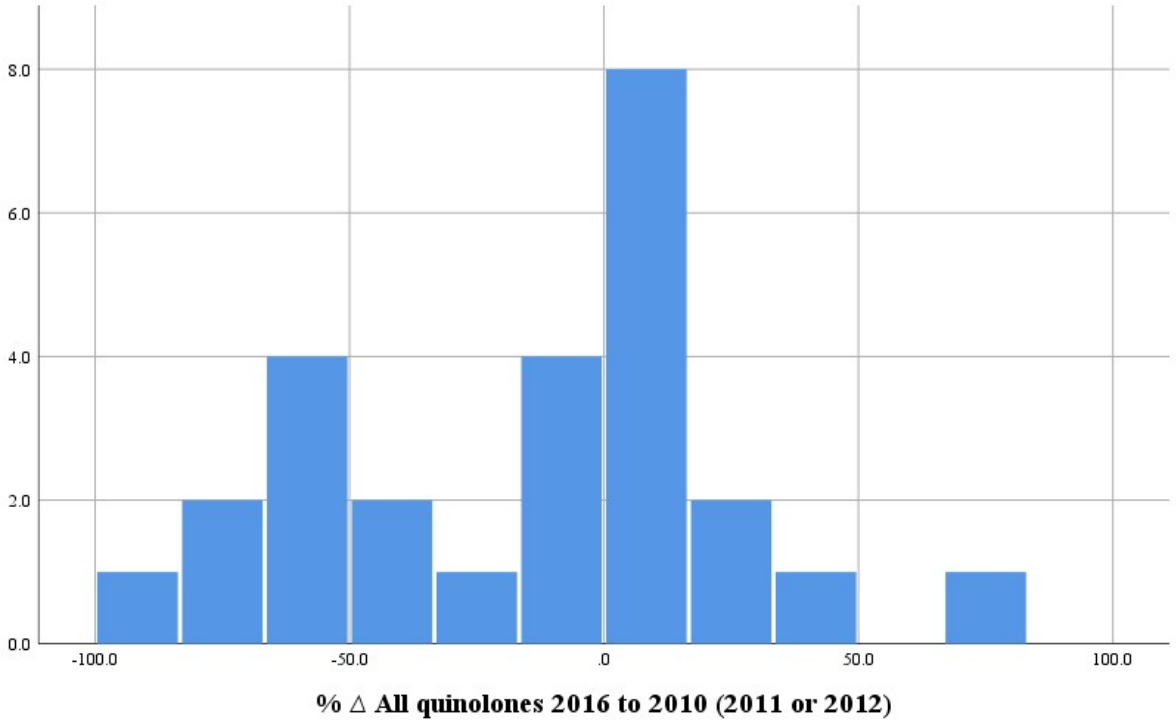


Figure 36. Variation on the percentage of all quinolones in mg/kg PCU, for the years 2010 to 2016. Fourteen countries had a decrease in their sales of quinolones, whilst 12 countries had an increase.

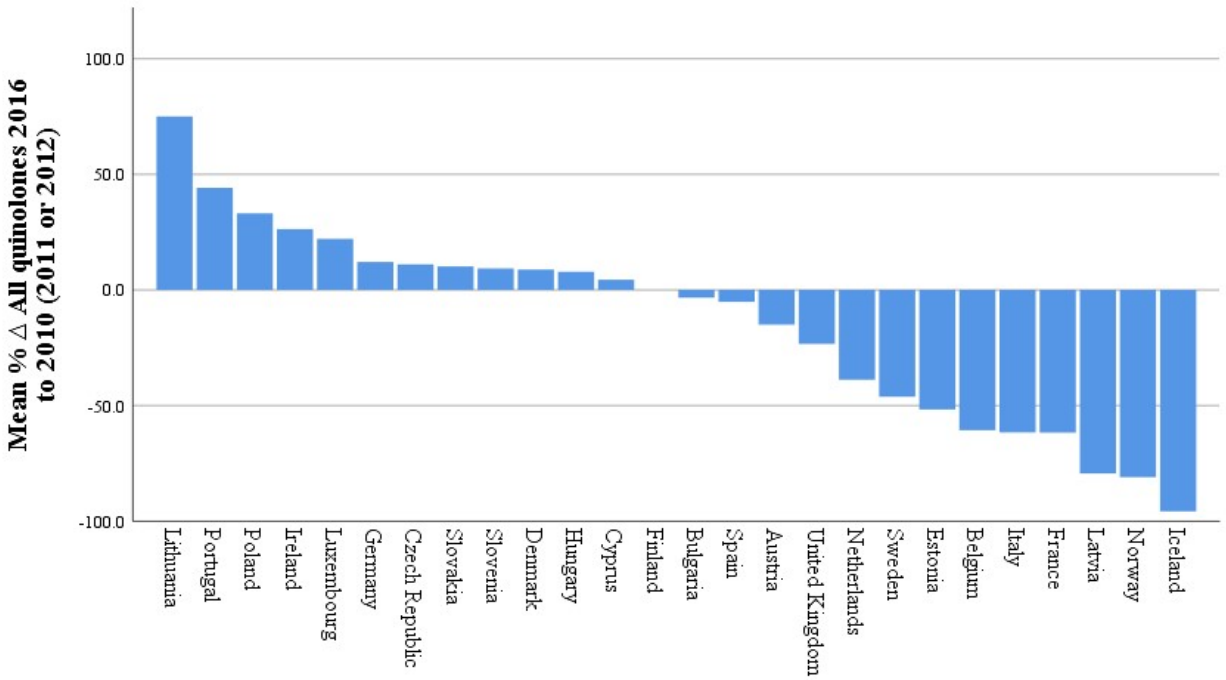


Figure 37. Variation in percentage of sales of quinolones (mg/kg PCU) between the years 2016 and 2010 (or 2011, 2012).

The following countries have decreased their sales of quinolones between the years 2010 (2011 or 2012) and 2016: Iceland (-95.8%), Norway (-81.0%), Latvia (-79.4%), France (-61.7%), Italy (-61.6%), Belgium (-60.0%), Estonia (-51.7%), Sweden (-46.2%), Netherlands (-38.8%), United Kingdom (-23.3%), Austria (-15.0%), Spain (-5.2%), Bulgaria (-3.0%) and Finland (0%),

Whilst others increased the sales during the same period: Lithuania (75.0%), Portugal (44.2%), Poland (33.1%), Ireland (26.3%), Luxembourg (22.1%), Germany (12.1%), Czech Republic (11.1%), Slovakia (10.2%), Slovenia (9.3%), Denmark (8.8%), Hungary (7.9%), and Cyprus (4.4%).

As in the case of the variation in the percentage of sales of 3rd and 4th generation cephalosporins, the increase of the use of quinolones in some countries is of concern considering that quinolones are classified as a HPCIA by the WHO [8, 155, 157-160] and Category B (Restrict) by the EMA/AMEG [10].

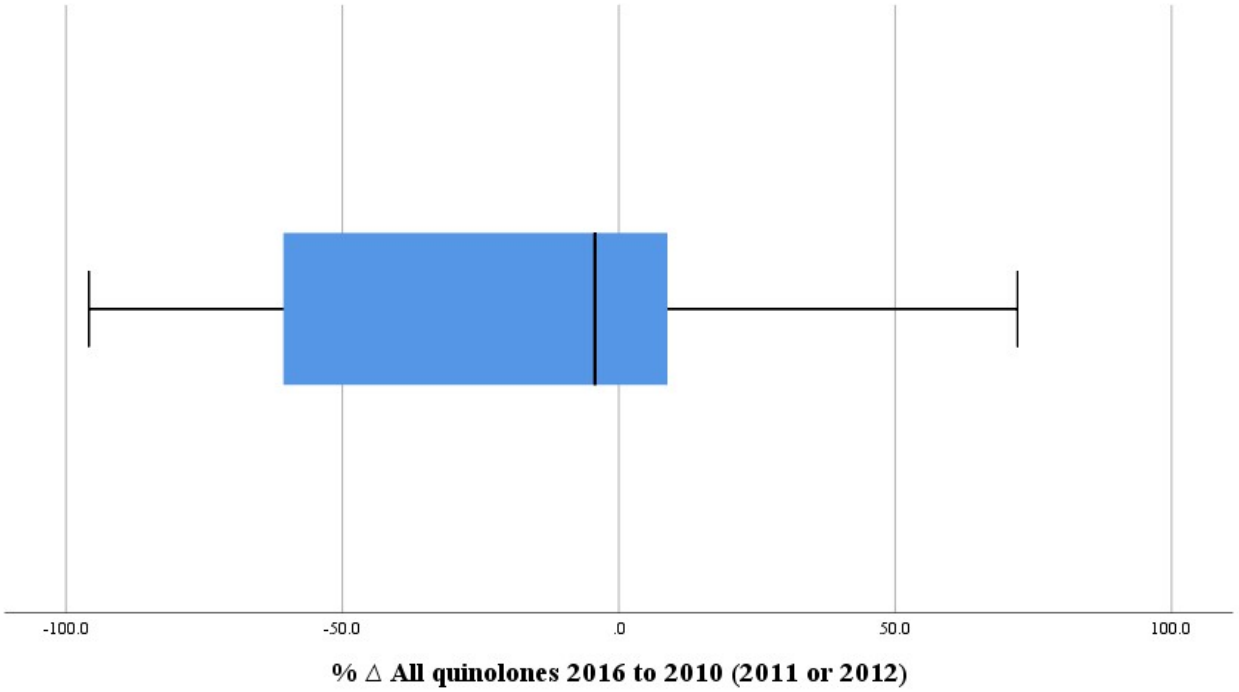


Figure 38. Box plot of variation in the percentage of quinolones between 2010 and 2016.

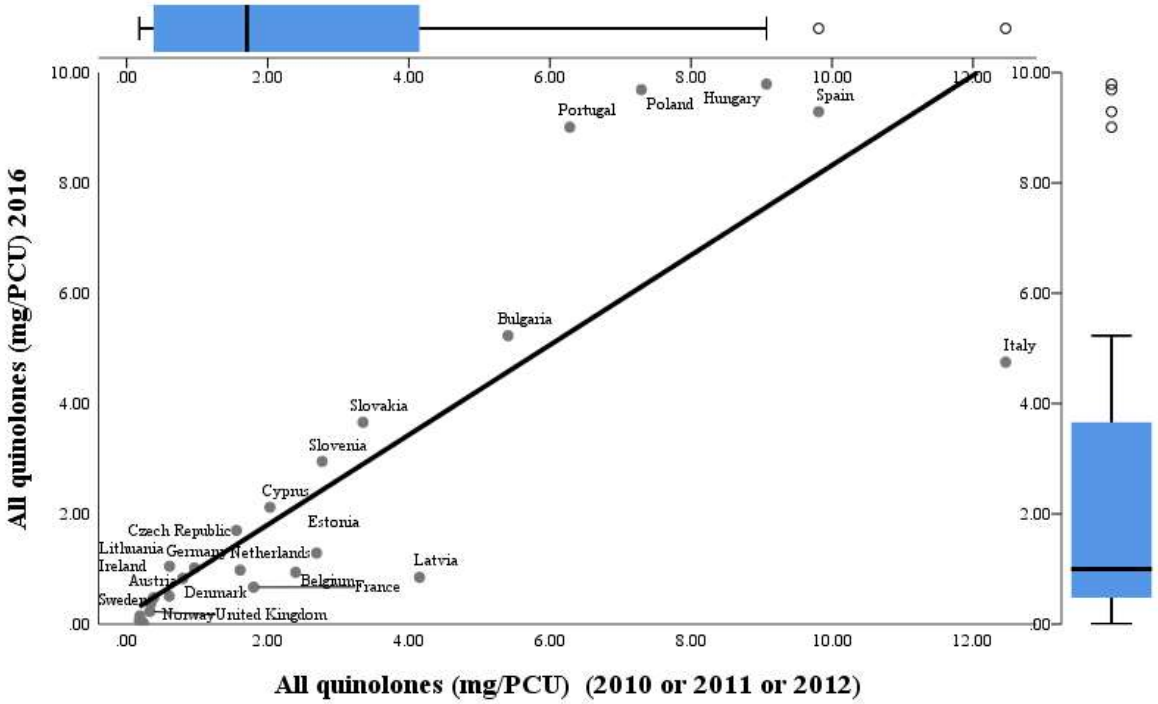


Figure 39. Quinolones in mg/kg PCU in 2016 *vs* 2010 (2011 or 2012 as appropriate).

The Pearson's linear correlation of sales of all quinolones in mg/kg PCU in 2010 (or 2011 or 2012) *vs* sales of quinolones in mg/kg PCU in 2016 is very strong (0.846) and significant at the 0.01 level (2-tailed), but not at the cut-off point of the Bonferroni correction.

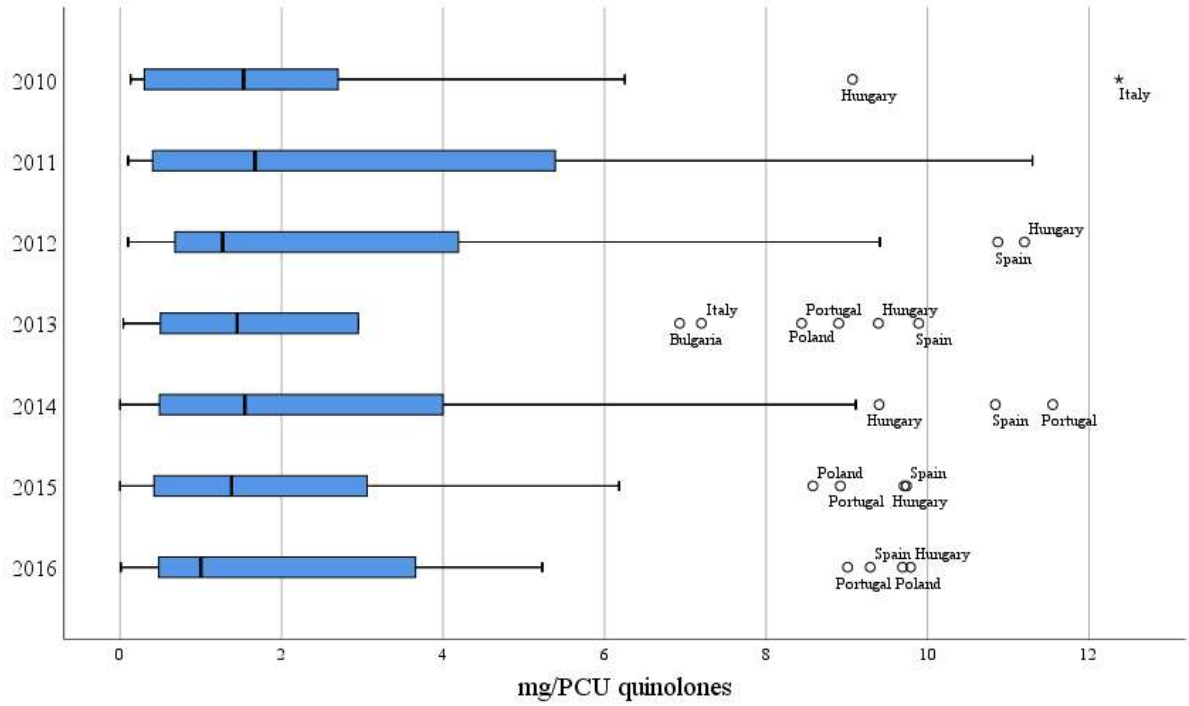


Figure 40. Variations in sales of quinolones between 2010 (or 2011, 2012) and 2016 in mg/kg PCU. The years in the graph are ordered in increasing order, with data from 2016 at the bottom of the graph.

9.2.2.1. Correlation of sales of quinolones in 2016 with the overall sales of antimicrobials in the country in 2016

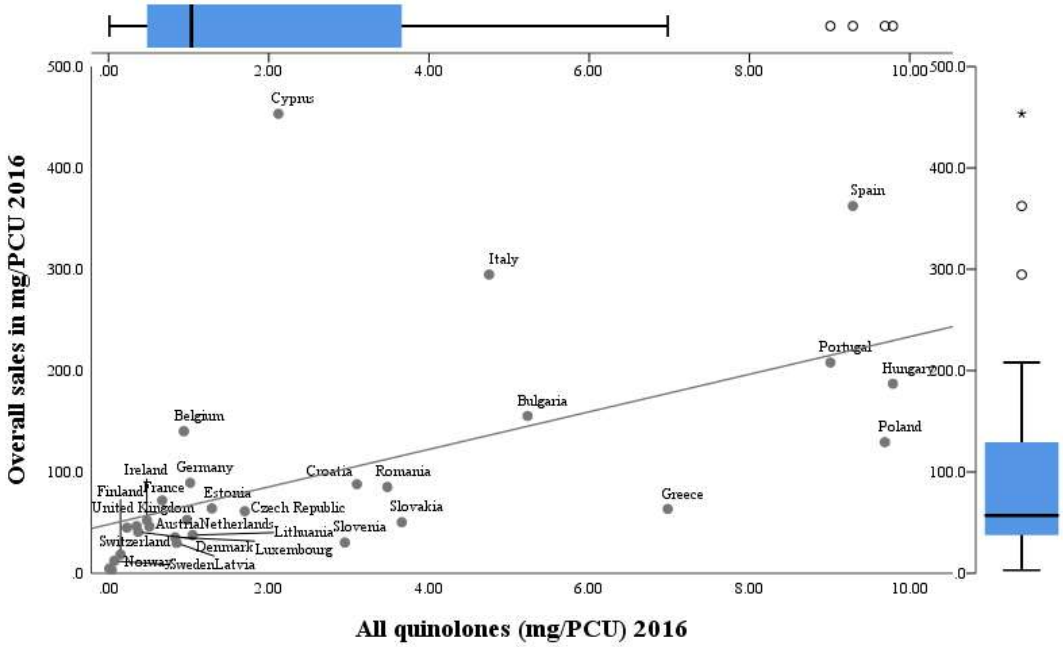


Figure 41. Overall sales of antimicrobials *vs* sales of quinolones in 2016.

The high sales of quinolones in Spain, combined with the high sales of all antimicrobials for use in food-producing species is of concern.

The Pearson's linear correlation of the overall sales of antimicrobials *vs* sales of quinolones in 2016 is moderate (0.551) and significant at the 0.01 level (2-tailed), but not at the cut-off point of the Bonferroni correction.

9.2.2.2. Other relevant correlations of quinolones

The Pearson's linear correlation detailed below are significant according to the Bonferroni correction (<0.00147 level, 2-tailed) (see 9.8.):

- The correlation of sales of quinolones in 2010 *vs* the overall sales of antimicrobials in 2010 is strong (0.664), as well as the sales of quinolones in 2010 *vs* the overall sales in 2016 (0.657).
- The correlation of polymyxins in 2016 *vs* the quinolones in 2016 is strong (0.693). Correlation of polymyxins in 2010 *vs* quinolones in 2010 is strong (0.768). Correlation of polymyxins in 2016 *vs* quinolones in 2010 is strong (0.792).

The above correlations of quinolones and polymyxins seem to indicate that the use of both classes of antimicrobials is strongly correlated.

- The sales of quinolones in 2010 are very strongly correlated with the sales of oral solutions (mg/kg PCU) in 2016 (0.864). The sales of quinolones in 2016 is strongly correlated with the sales of oral solutions in 2016, which seem to indicate that quinolones are used mostly orally.
- Sales of quinolones in 2010 are correlated to the total sales in tonnes in 2016 (0.615).
- Sales of quinolones in 2016 are moderately correlated with the percentage of poultry in 2016 (0.570).
- Sales of quinolones in 2016 are strongly correlated with the average high temperature (0.611).

9.2.3. Results of sales of polymyxins

Contrary to other values discussed in this report, the data precision for the sales of polymyxins the ESVAC online tool[2] is lower than for other categories, i.e. when the data are downloaded those data are provided with only one decimal, and when sales are below 1 mg/kg PCU the results indicate *"<1 mg/kg PCU*" and not the detailed value. This is for confidential reasons as some countries only have one or two products containing polymyxins authorised.

Most of the sales of the group polymyxins are colistin [65].

In order to analyse the data on this important group of antimicrobials the data was mostly downloaded from the ESVAC database (as for the category of 3rd and 4th generation cephalosporins and quinolones), but where on the ESVAC tool indicates *"<1 mg/kg PCU"* the data was obtained from a combination of the data from the ESVAC printed report (years 2015 and 2016) or by multiplying the % of mg/kg PCU sold by the overall sales of antimicrobials in the year in mg/kg PCU (years 2010 to 2014), depending of the data provided. For the year 2015 and 2016 data for Ireland were not available in detail for confidential reasons and were not included in the estimations, those sales were in any case below 1 mg/kg PCU.

[2] Available at https://www.ema.europa.eu/en/veterinary-regulatory/overview/antimicrobial-resistance/european-surveillance-veterinary-antimicrobial-consumption-esvac

Table 12. Sales of polymyxins in mg/kg PCU, for the years 2010 to 2016 including the variation in the percentage of sales between the years 2010 and 2016*.

Country	Polymyxins§							Percentage polymyxins mg/kg PCU, years 2010 to 2016*
	2010	2011	2012	2013	2014	2015	2016	
Austria	0.9	1.0	0.7	0.9	1.6	1.6	1.6	77.8%
Belgium	5.9	5.3	5.7	4.7	3.3	2.7	2.4	-59.3%
Bulgaria	NA	3.1	3.7	2.7	0.5	3.6	2.3	-25.8%
Croatia	NA				3.8	2.4	3.5	Not computed
Cyprus	NA	8.1	8.1	8.4	11.1	12.3	11.1	37.0%
Czech Republic	0.9	0.5	0.9	1.1	1.0	1.0	0.8	-11.1%
Denmark	0.2	0.2	0.2	0.2	0.4	0.5	0.5	***150%***
Estonia	3.5	4.3	4.8	5.7	3.1	1.3	0.7	***-80.0%***
Finland	No sales of polymyxins							
France	8.7	7.8	6.7	5.9	7.0	4.0	2.8	-67.8%
Germany	NA	14.8	14.8	14.6	12.21	9.1	7.9	-46.6%
Greece	NA					3.3	1.0	Not computed
Hungary	6.8	8.9	7.8	10.0	7.0	9.6	12.2	79.4%
Iceland	No sales of polymyxins							
Ireland	NA	***0.1***	***0.1***	***0.1***	***0.1***	NA	NA	Not computed
Italy	***40.1***	30.6	***30.0***	***27.5***	29.4	26.1	15.1	-62.3%
Latvia	1.0	1.0	2.5	1.5	0.8	0.9	0.9	-10.0%
Lithuania	NA	1.4	1.3	***0.1***	***0.1***	0.6	1.0	-28.6%
Luxembourg	NA		1.7	3.1	2.4	1.4	1.0	-41.2%
Netherlands	NA	1.5	1.0	0.6	0.5	0.5	0.3	***-80.0%***
Norway	No sales of polymyxins							

Country	Polymyxins[§]							Percentage polymyxins mg/kg PCU, years 2010 to 2016*
	2010	2011	2012	2013	2014	2015	2016	
Poland	NA	4.1	3.9	4.4	5.0	5.9	5.6	36.6%
Portugal	15.1	7.9	18.6	18.9	17.5	14.6	13.5	-10.6%
Romania	NA				6.4	7.4	5.5	Not computed
Slovakia	NA	1.2	2.1	1.1	1.5	1.1	1.1	-8.3%
Slovenia	***0.1***	***0.1***	***0.1***	***0.05***	***0.1***	***0.1***	***0.1***	0%
Spain	NA	***33.4***	29.3	21.4	***36.1***	***34.9***	***22.0***	-34.1%
Sweden	***0.1***	***0.1***	***0.1***	***0.1***	***0.1***	***0.1***	***0.1***	0%
Switzerland	1.8	1.8	1.3	1.1	0.9	0.6	0.5	-72.2%
United Kingdom	***0.1***	***0.1***	***0.1***	***0.1***	***0.1***	***0.1***	***0.02***	***-80.0%***

*For some countries (Croatia, Greece and Romania) data were not collected until after the year 2013, and the percentage of change was not computed.
For Bulgaria, Cyprus, Germany, the Netherlands, Poland and Slovakia the data are from the year 2011, and for Luxembourg, 2012.
§ NA: Not available
Blue, bold and italics highlight sales below 0.1 mg/kg and/or the lower result or percentage value per column. Red, bold and italics the highest.

Finland, Iceland and Norway have no sales of polymyxins, the lowest sales of polymyxins per mg/kg PCU were for Slovenia, Sweden and the United Kingdom, where sales were below 0.1 mg/kg PCU for all the years. Ireland where sales were also below 0.1 mg/kg PCU for the years for which data are available, Slovenia for which all the sales were below 0.1 mg/kg PCU (0.05 in the year 2013) and for Lithuania in 2013 and 2014 where sales were also below 0.1 mg/kg PCU.

The highest sales of polymyxins in mg/kg PCU were for Italy (40.1 year 2010, 30.0 year 2012 and 27.5 year 2013) and Spain (33.4 year 2011, 36.1 year 2014, 34.9 year 2015 and 22.0 year 2016).

The biggest decrease of sales of polymyxins sold per mg/kg PCU was for Estonia, the Netherlands and United Kingdom (-80%) and the highest increase for Denmark (150%). The latest ESVAC report [71] indicates that sales of polymyxins are generally low in Denmark (0.5 mg/kg PCU in 2016) and that sales are expected to decrease in the future due to a new *yellow card* initiative, Denmark is the 9th lowest value of the 30 countries reporting sales data to the ESVAC project. The FAO report on tackling AMR in pigs [206] indicates that following the EMA recommendations on colistin the Danish Government increased the multiplication factor for colistin in the Yellow Card Initiative and that the use of colistin for pigs has since been almost zero.

The many factors that influence the data collection, especially the need to gain experience by countries that are starting the collection of such data causes that the indicator of variation of sales

between the years 2010 and 2016 (in percentage) might not be as robust as desirable, especially in cases were the use of antimicrobials is small or the detail of the data available, limited.

Table 13. Overall sales statistics of polymyxins in mg/kg PCU in years 2010 to 2016[§].

		Polymyxins (mg/kg PCU)						
		2010	2011	2012	2013	2014	2015	2016
Valid countries		17	26	27	27	29	29	29
Missing countries*		13	4	3	3	1	1	1
Mean		5.0	5.3	5.4	5.0	5.3	5.0	3.9
Std. Deviation		10.0	8.7	8.4	7.4	8.8	8.2	5.6
Minimum		0	0	0	0	0	0	0
Maximum		40.2	33.5	30.1	27.6	36.1	34.9	22.0
Percentiles	25	0.1	0.1	0.1	0.1	0.1	0.5	0.4
	50	1.0	1.5	1.7	1.1	1.5	1.5	1.1
	75	6.5	7.8	6.8	5.9	6.8	6.7	5.6

[§] Including countries in the database with zero sales of polymyxins.
[*]Countries not included by year:
2010-2016: Finland, Iceland and Norway, no sales for any of the years.
2010: Bulgaria. Croatia, Cyprus, Greece, Germany, Ireland, Lithuania, Luxemburg, the Netherlands, Romania, Slovakia, Spain and Switzerland.
2011: Croatia, Greece, Luxembourg and Romania.
2012 and 2013: Croatia, Greece and Romania.
2014: Greece.
2015 and 2016: Ireland.

The variation in the percentage of polymyxins expressed as mg/kg PCU of the years 2010 to 2016 of the countries providing data to ESVAC (years 2010 to 2016) shows a decrease on the sales of those polymyxins of -13.0% it should be noted that the range of variation is high (std = 55.3%).

The percentage variation of polymyxins expressed as mg/kg PCU between 2010 (2011 or 2012) (5.73) and 2016 (3.9), by one-sample t-test, is not statistically different from zero ($p = 0.243$).

As indicated, for the above statistical analysis the countries in which there are no sales of polymyxins (Finland, Iceland and Norway) were included, the same statistical analysis was repeated excluding the countries with no sales of polymyxins.

Table 14. Overall sales of polymyxins in mg/kg PCU in the years 2010 to 2016, excluding countries in which there are no sales of polymyxins.

		Polymyxins (mg/kg PCU)						
		2010	2011	2012	2013	2014	2015	2016
Valid countries		14	23	24	24	26	26	26
Missing countries*		16	7	6	6	4	4	4
Mean		6.1	6.0	6.1	5.6	5.9	5.6	4.4
Std. Deviation		10.7	9.1	8.7	7.7	9.1	8.4	5.8
Minimum		0.1	0.1	0.1	0.0	0.1	0.1	0.0
Maximum		40.2	33.5	30.1	27.6	36.1	34.9	22.0
Percentiles	25	0.3	0.6	0.8	0.3	0.5	0.6	0.7
	50	1.4	1.8	2.3	2.1	2.0	2.0	1.4
	75	7.4	7.9	7.6	7.8	7.1	7.9	6.2

*Countries not included by year:
2010-2016: Finland, Iceland and Norway, no sales for any of the years.
2010: Bulgaria. Croatia, Cyprus, Greece, Germany, Ireland, Lithuania, Luxemburg, the Netherlands, Romania, Slovakia, Spain and Switzerland.
2011: Croatia, Greece, Luxembourg and Romania.
2012 and 2013: Croatia, Greece and Romania.
2014: Greece.
2015 and 2016: Ireland.

The variation in the percentage of polymyxins expressed as mg/kg PCU of the years 2010 to 2016 of 24 countries providing data to ESVAC (years 2010 to 2016, excluding those with no sales) shows a decrease on the sales of those polymyxins of -14.7% it should be noted that the range of variation is high (std = 58.7%).

The percentage variation of polymyxins expressed as mg/kg PCU between 2010 and 2016, by one-sample t-test is not statistically different from zero ($p = 0.244$).

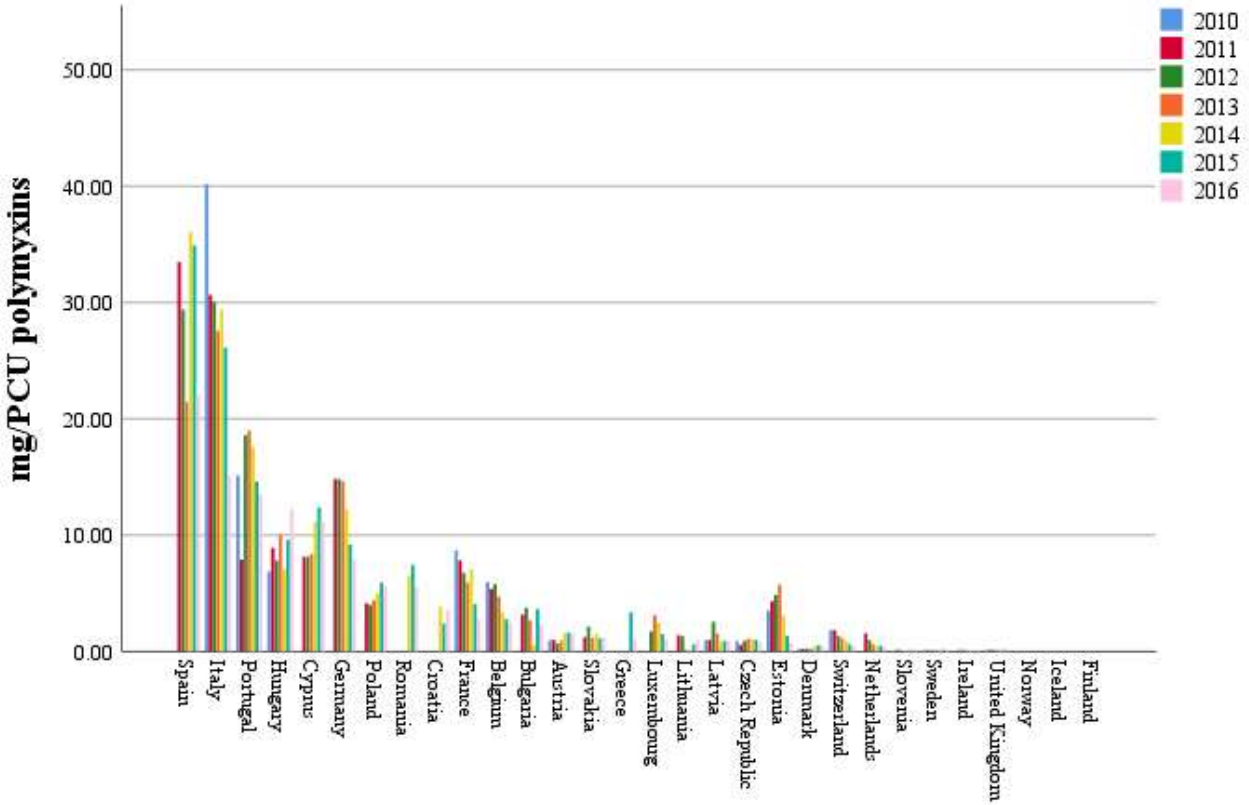


Figure 42. Overall sales of polymyxins in mg/kg PCU for all years (2010 to 2016) for all countries reporting data to ESVAC, ordered by sales (decreasing) in the year 2016.

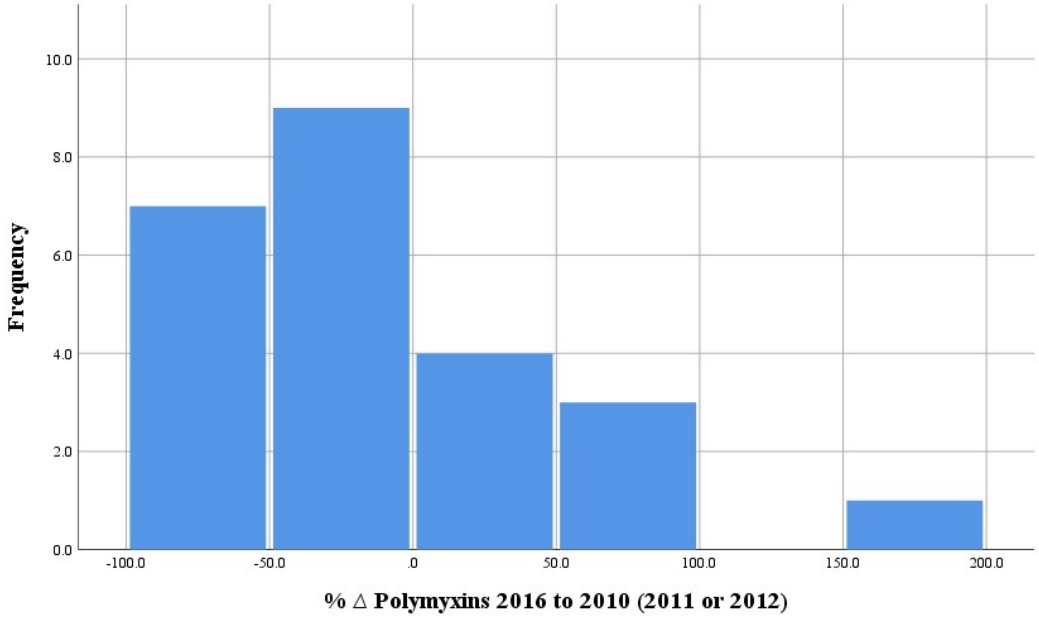


Figure 43. Variation in decrease and increase in the percentage of 3rd and 4th generation cephalosporins between 2016 and 2010 (or 2001, 2012), excluding countries in which there are no sales of polymyxins (Finland, Iceland and Norway).

In the period between the year 2010 (2011 or 2012), 16 countries have decreased their sales of antimicrobials, whilst 8 countries have increased such sales.

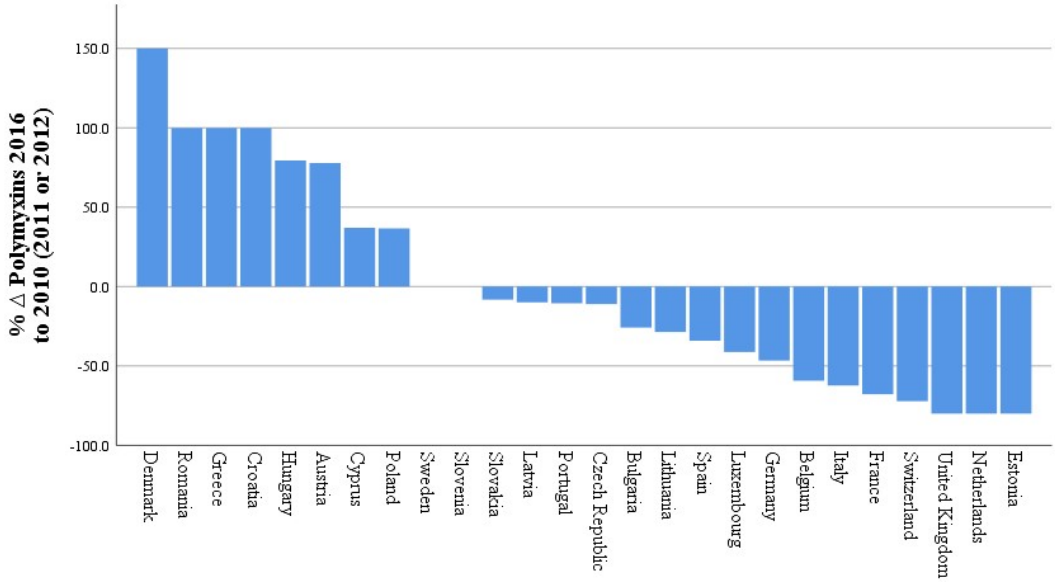


Figure 44. Variation in the percentage of sales of polymyxins between the years 2010 (2011 or 2012 as appropriate) and 2016 (26 countries) by decreasing percentage of sales, excluding countries in which there are no sales of polymyxins (Finland, Iceland and Norway).

As already indicated above, although it seems that there is a substantial increase of sales for Denmark between the studied years (150%), it has to be noted that the sales in mg/PUC of polymyxins in Denmark are very low (0.5 mg/kg PCU) and below the EMA/CVMP recommendations on polymyxins [114], therefore their increase in total amount of use of polymyxins is minimal.

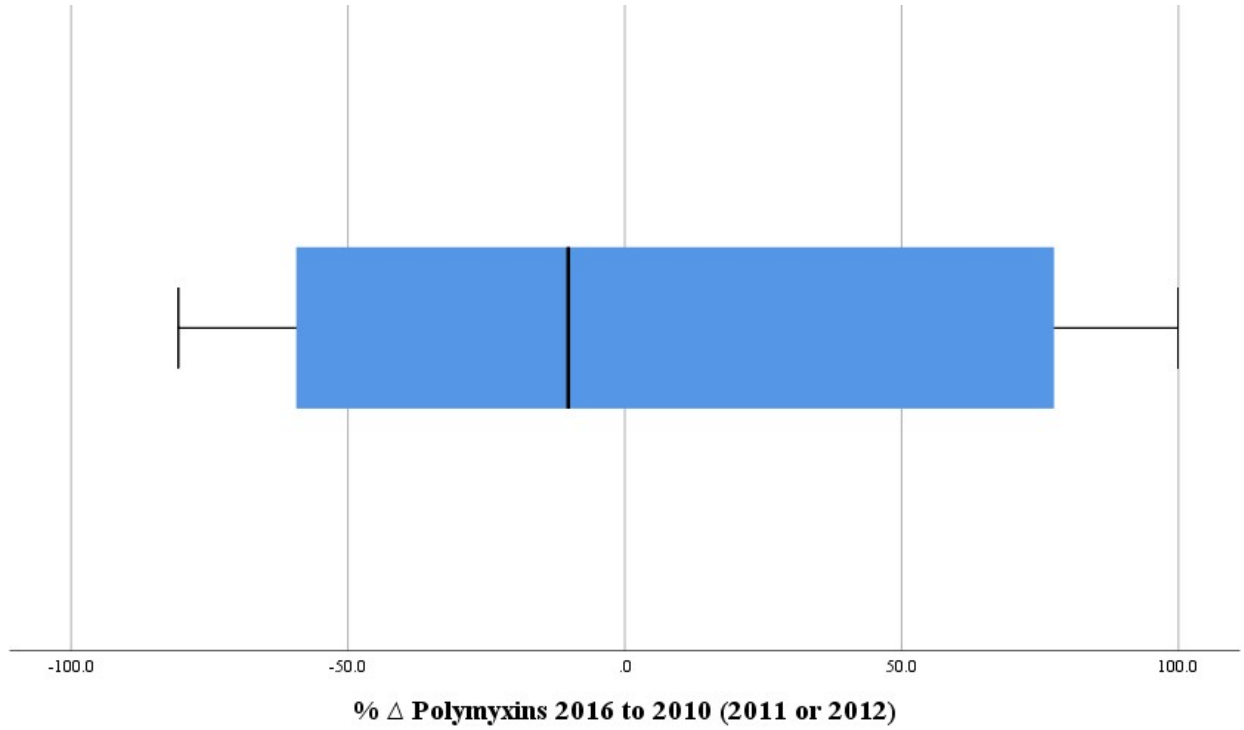


Figure 45. Percentage of variation of polymyxins in mg/kg PCU between 2010 (2011 or 2012) and 2016 of 24 countries providing data to ESVAC.

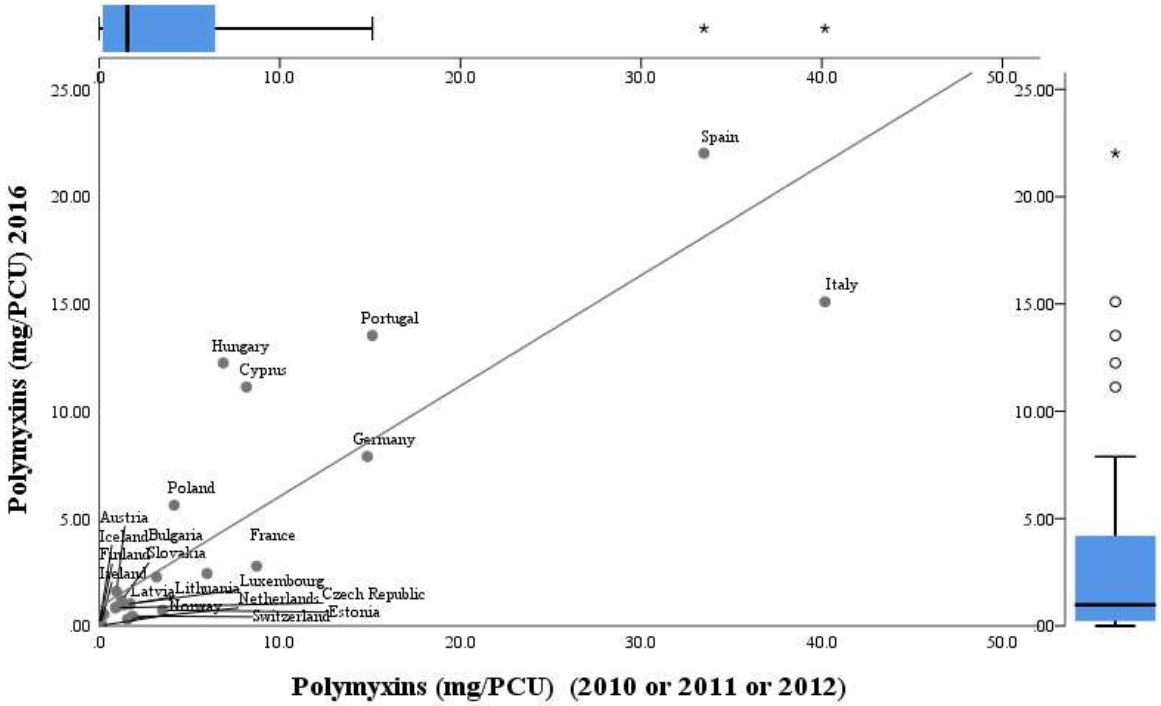


Figure 46. Polymyxins in mg/kg PCU in 2016 *vs* 2010 (2011 or 2012 as appropriate).

For the sake of clarity, the scales of the x and y axes of the previous graph are different (25 *vs* 50 mg/kg PCU), allowing to better appreciate the decrease in the use of polymyxins between 2010 and 2016. It can be seen that countries that have a high general consumption of antimicrobials exhibit a different behaviour between 2010 and 2016. While some reduce the use of polymyxins (expressed in mg/kg of PCU), others increase it.

According to Table 4 and Table 12, Spain has an overall increase in sales of all antimicrobials of 39.7% but reduction of sales of polymyxins of 34.1% during the same period, Portugal has an overall increase of 16.9% of sales of all antimicrobials expressed as mg/kg PCU and a polymyxins decrease of -10.6%, Hungary has an overall decrease of 30.7% of sales of all antimicrobials and a polymyxins increase of 79.4%, Cyprus has an overall increase of 11.2% of sales of all antimicrobials and an increase of polymyxins of 37.0%, Poland has an overall increase of 1.6% sales of antimicrobials and an increase in sales of polymyxins of 36.6%. The case of Italy is contrary to those since although it reduces the general use of antimicrobials in the period by 30.0%, it also reduces the sales of polymyxins by 62.3%. It is to be noted that some of the countries mentioned are taking decisive action to reduce the use of polymyxins.

The Pearson's linear correlation of sales of polymyxins in mg/kg PCU in 2010 (or 2011 or 2012) *vs* sales polymyxins in 2016 is very strong (0.872) and significant at the cut-off point of the Bonferroni correction (<0.00147 level, 2-tailed).

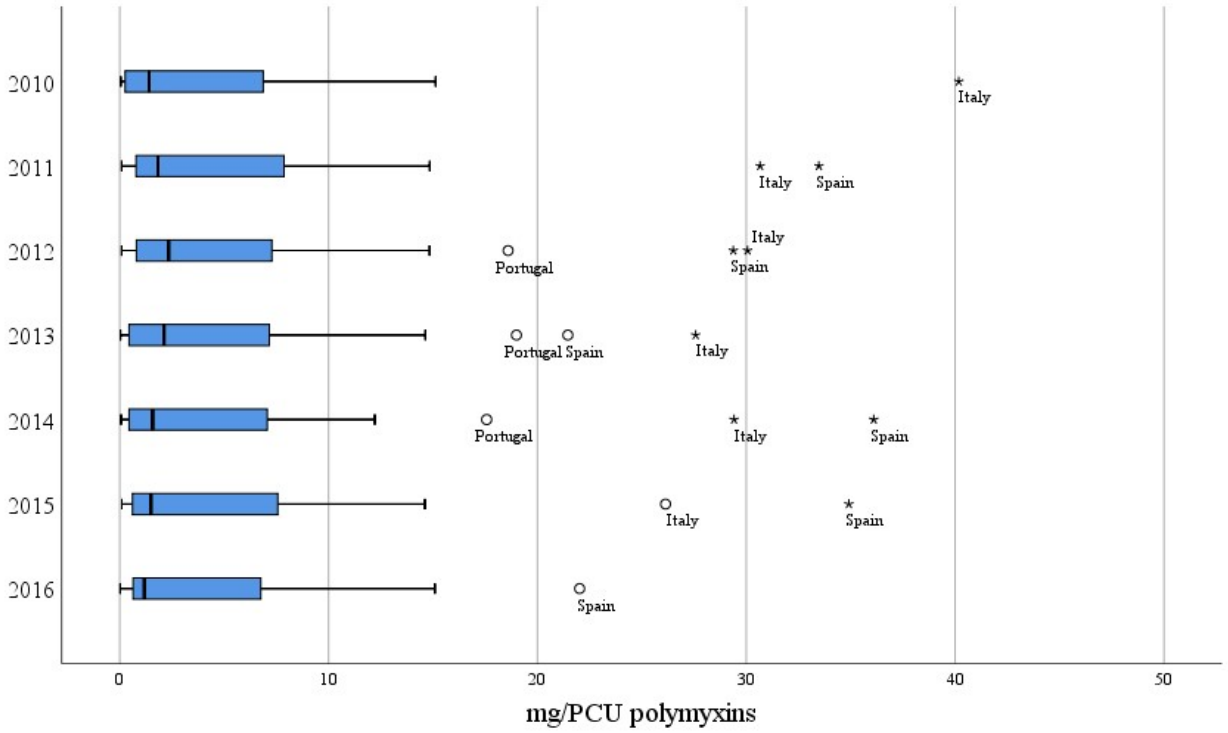


Figure 47. Variations in sales of polymyxins between 2010 (or 2011, 2012) and 2016 in mg/kg PCU. The years in the graph are ordered in increasing order, with data from 2016 at the bottom of the graph.

For most years, the sales in mg/kg PCU of polymyxins in Spain and Italy are more than 3 times the interquartile range considering all countries, whist the value for Portugal exceeds 1.5 times that range in some years.

9.2.3.1. Correlation of sales of polymyxins in 2016 with the overall sales of antimicrobials in the country in 2016

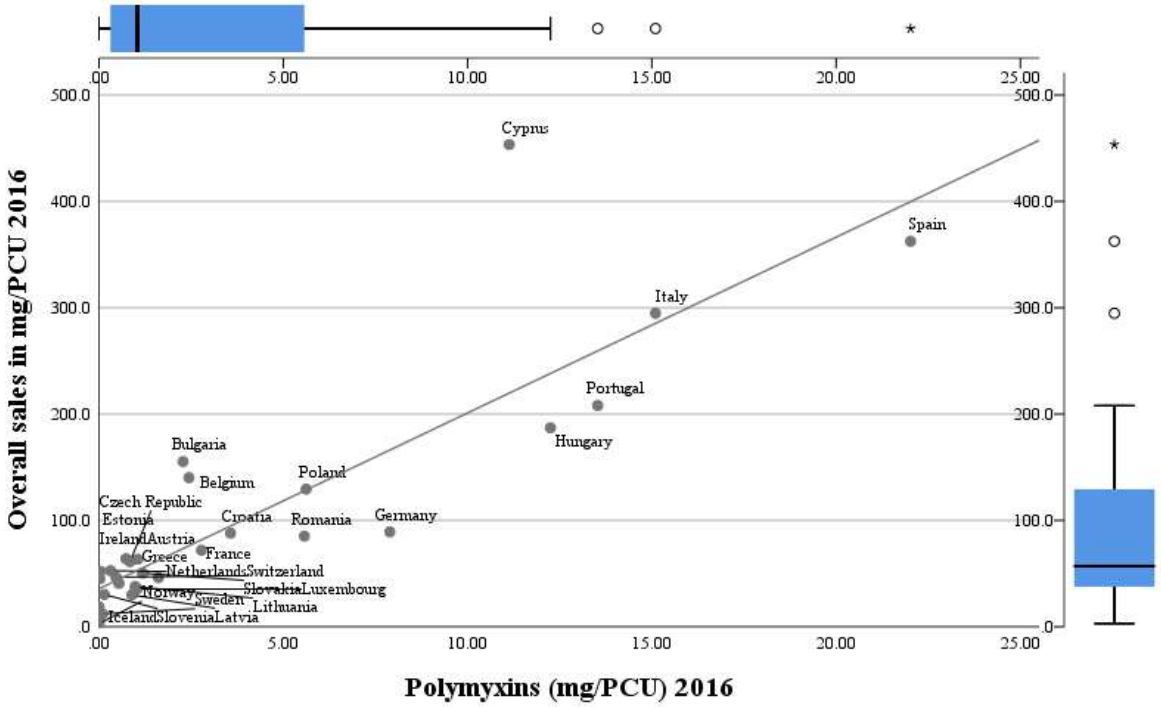


Figure 48. Overall sales of antimicrobials *vs* sales of polymyxins in 2016.

Sales on polymyxins in 2016 are very strongly correlated with the overall sales in 2016 (0.861) and significant at the cut-off point of the Bonferroni correction (<0.00147 level, 2-tailed).

9.2.3.2. Other relevant correlations of polymyxins

The Pearson's linear correlations detailed below are significant according to the Bonferroni correction (<0.00147 level, 2-tailed) (see 9.8.).

- In addition to the very strong correlation of sales of polymyxins in 2016 with the overall sales in 2016. The sales of polymyxins in 2016 are also very strongly correlated with the overall sales of antimicrobials in 2010 (0.819). Sales of polymyxins in 2010 are strongly correlated with the sales of antimicrobials in 2010 (0.773) and with the sales of antimicrobials in 2016 (0.719).

As already noted above (see 9.2.2.2.) sales of polymyxins are correlated to sales of quinolones.

- Sales of polymyxins in 2016 are strongly correlated with the sales in tonnes of antimicrobials in 2016 (0.776). Sales of polymyxins in 2010 are in addition very strongly correlated with the total sales in tonnes in 2016 (0.836) (no logical explanation could be found for such stronger correlation with the year 2010 than with the year 2016).
- Sales of polymyxins in 2016 are strongly correlated with the sales of premix in 2016 (0.761), and with the percentage of premixes in 2016 (0.614).
- Sales of polymyxins in 2010 are strongly correlated with the sales of oral solutions in 2016 (0.759) (as above, no logical explanation could be found for such correlation).

- Sales of polymyxins in 2016 are strongly correlated with the sales of oral solutions in 2016 (0.734).
- Sales of polymyxins in 2016 are strongly correlated with the average temperature (0.664), with the average high temperature (0.684) and with the average low temperature (0.640). Similar correlations can be found with the sales of polymyxins in 2010 and the temperature (see Table 36).

9.2.4. EMA recommendations on polymyxins

In a document dated July 2016, the EMA recommended that colistin-containing medicines should only be used as a second-line treatment in animals and that their sales should be minimised across all EU [114].

The advice indicates that for the "high and moderate" consumers, the target and desirable levels are set at 5 and 1 or below 1, mg/kg PCU, respectively. This estimation is based on the observations on the level of use in other countries, i.e. it is a combination of the experience of the countries using colistin *vs* countries not using colistin, combined with setting targets that are feasible. The advice also indicates that reduction in the use of colistin should be achieved without an increase in the use (in mg/kg PCU) of fluoroquinolones, 3rd and 4th generation cephalosporins or overall consumption of antimicrobials.

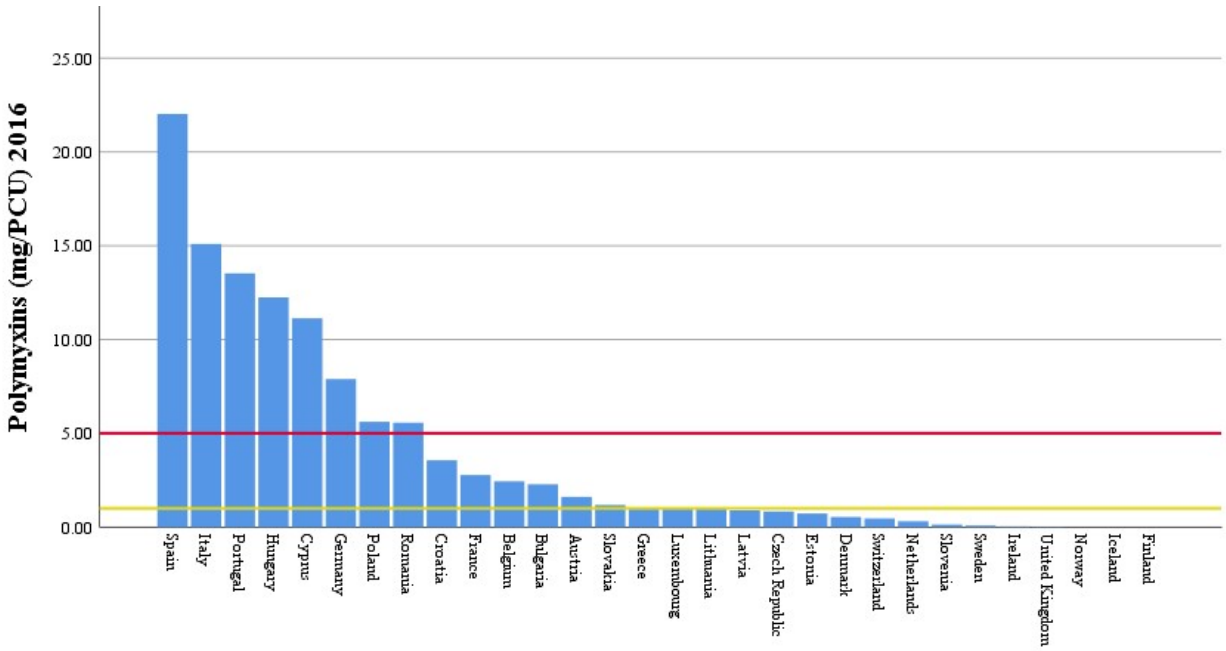


Figure 49. Overall sales of polymyxins in 2016. Red line at 5 mg/kg PCU, the yellow line at 1 mg/kg PCU.

As the recommendation is dated July 2016, it is still too early to analyse if the recommendation to decrease the use of colistin has been followed by the EU MSs. In any case, the current situation is as follows: 3 countries reported no sales of colistin: Finland, Iceland and Norway. Thirteen countries reported sales in 2016 below the desirable value of 1 mg/kg PCU (in mg/kg PCU): United

Kingdom (0.02), Slovenia (0.1), Sweden (0.1), Netherlands (0.3), Denmark (0.5), Switzerland (0.5), Estonia (0.7), Czech Republic (0.8), Latvia (0.9), Ireland (below 1), Greece (1.0), Lithuania (1.0) and Luxembourg (1.0). Between the recommended value of 5 mg/kg PCU and the desirable value of 1 mg/kg PCU (in mg/kg PCU): Slovakia (1.1), Austria (1.6), Bulgaria (2.3), Belgium (2.4), France (2.8) and Croatia (3.5). Eight countries reported sales above the recommended value of 5 mg/kg PCU (in mg/kg PCU): Romania (5.5), Poland (5.6), Germany (7.9), Cyprus (11.1), Hungary (12.2), Portugal (13.5), Italy (15.1) and Spain (22.0).

In summary, during 2016, eight of the 30 countries reporting data to ESVAC in 2016 were above the recommended 5 mg colistin per kg PCU, and 15 above the desirable 1mg colistin per kg PCU.

9.3. Results of sales of antimicrobials (as mg/kg PCU) of oral forms for group treatment

The analysis of the pharmaceutical form in which the antimicrobials are given to the animals as one of the reasons for high or low consumption is of interest to try to reduce antimicrobial use.

Antimicrobials can be provided in many pharmaceutical forms to animals; the ESVAC project has simplified those forms into; premix, oral powder, oral solution, injection, oral paste, bolus, intramammary preparations, intrauterine preparations and tablets.

Of those pharmaceutical forms, the premix, oral-powder and oral-solutions are most likely used for group treatment. Injectable forms will also be used for groups of animals but have to be administered individually which makes it more laborious, the same is true for the other forms; oral paste, bolus, intramammary and intrauterine preparations.

Tablets are excluded from the calculations as those are likely to be given to companion animals which are not included in the animal biomass (or PCU), those forms would usually be identified as “oral treatments”.

The percentage of oral forms for group treatment is calculated by adding the tonnes of the 3 main forms administered as group treatment, i.e. premix, oral powder and oral solution. The forms of oral paste, bolus and tablets were disregarded from this calculation as although they are administered orally, they are administered individually to the animals.

The ESVAC report [64, 65, 70, 71, 189] publishes in Table A2 of the Annex 1 the “Distribution of sales, in mg/kg PCU, of veterinary antimicrobial agents applicable mainly for food-producing animals, including horses, by administration route/form and country for 2016”.

To calculate the percentage of the oral forms for group treatment, the mg/kg PCU in 2016 of premix, oral-powder and oral solutions were added and divided by total (all other forms) mg/kg PCU in 2016.

Table 15. Distribution of sales, in mg/kg PCU, of veterinary antimicrobial agents applicable for food-producing animals, by administration route/form and country, including the percentage of oral forms for group treatment for 2016.

Country	Sales in mg/kg PCU, year 2016									Percentage of oral forms for group treatment
	Premix	Oral powder	Oral solution	Injection	Oral paste	Bolus	Intramammary preparations	Intrauterine	Total	
Austria	2.0	35.6	1.0	5.9	0.3	NS*	1.2	0.1	46.1	83.7%
Belgium	21.	101.1	3.7	13.7	NS	0.1	0.4	0.2	140.1	89.7%
Bulgaria	65.6	14.6	64.4	9.9	NS	NS	0.8	0.1	155.3	93.1%
Croatia	8.8	31.9	27.3	18.1	NS	0.9	0.6	0.4	87.9	77.3%
Cyprus	358.7	58.3	16.4	19.1	0.1	0.2	0.7	NS	453.4	95.6%
Czech Republic	9.3	14.7	27.4	8.3	NS	NS	1.1	0.5	61.2	83.8%
Den-mark	0.6	4.2	20.0	15.2	0.5	NS	0.2	0.1	40.8	60.6%
Estonia	NS	42.8	1.4	18.1	NS	NS	1.5	0.2	64.	69.1%
Finland	2.9	4.4	0.	9.7	1.1	NS	0.5	-	18.6	39.5%
France	27.9	1.	28.9	12.9	0.1	0.1	1.	0.1	71.9	80.3%
Germany	0.1	42.	39.4	6.2	0.2	0.	0.7	0.6	89.2	91.4%
Greece	33.3	NS	21.3	8.8	NS	NS	0.1	NS	63.5	86.0%
Hungary	96.1	39.7	44.4	6.4	NS	NS	0.2	0.2	187.1	***96.4%***
Iceland	NS	0.1	0.1	4.	0.1	NS	0.3	0.1	4.7	***4.5%***
Ireland	17.6	7.7	9.8	14.2	NS	0.3	2.5	NS	52.1	67.3%
Italy	116.8	43.7	116.3	17.1	0.2	NS	0.5	0.2	294.8	93.9%
Latvia	NS	6.4	9.6	11.2	NS	NS	1.3	1.4	29.9	53.7%

Country	Sales in mg/kg PCU, year 2016									Percentage of oral forms for group treatment
	Premix	Oral powder	Oral solution	Injection	Oral paste	Bolus	Intramammary preparations	Intrauterine	Total	
Lithuania	0.2	20.3	5.9	7.7	NS	1.3	2.1	0.2	37.7	70.0%
Luxembourg	NS	15.	6.9	12.2	0.2	0.1	0.9	0.3	35.5	61.5%
Netherlands	0.4	2.7	40.6	8.1	0.3	NS	0.5	0.1	52.7	82.9%
Norway	0.1	0.1	0.1	1.8	0.7	NS	0.1	0.1	2.9	9.3%
Poland	8.4	0.5	105.9	11.9	NS	NS	2.5	0.2	129.4	88.7%
Portugal	123.3	10.1	62.7	11.3	NS	NS	0.5	NS	208.	94.3%
Romania	7.8	0.6	63.9	12.7	NS	0.1	0.1	0.1	85.2	84.8%
Slovakia	9.9	2.6	28.6	8.5	NS	NS	0.7	0.1	50.4	81.6%
Slovenia	0.3	10.7	9.3	8.7	NS	NS	1.0	0.4	30.3	66.8%
Spain	248.2	NS	98.5	15.6	NS	NS	0.1	NS	362.5	95.7%
Sweden	0.3	0.1	0.9	9.2	1.5	NS	0.2	NS	12.1	10.8%
Switzerland	26.	5.6	0.1	10.3	0.5	NS	3.2	0.9	46.6	68.0%
United Kingdom	20.7	4.5	9.0	10.	0.1	0.2	0.5	NS	45.0	75.8%

*NS: No sales superior to 0.1 mg/kg PCU

In the column “Percentage of oral forms for group treatment “, blue, bold and italics highlight the lower percentage value. Red, bold and italics the highest percentage.

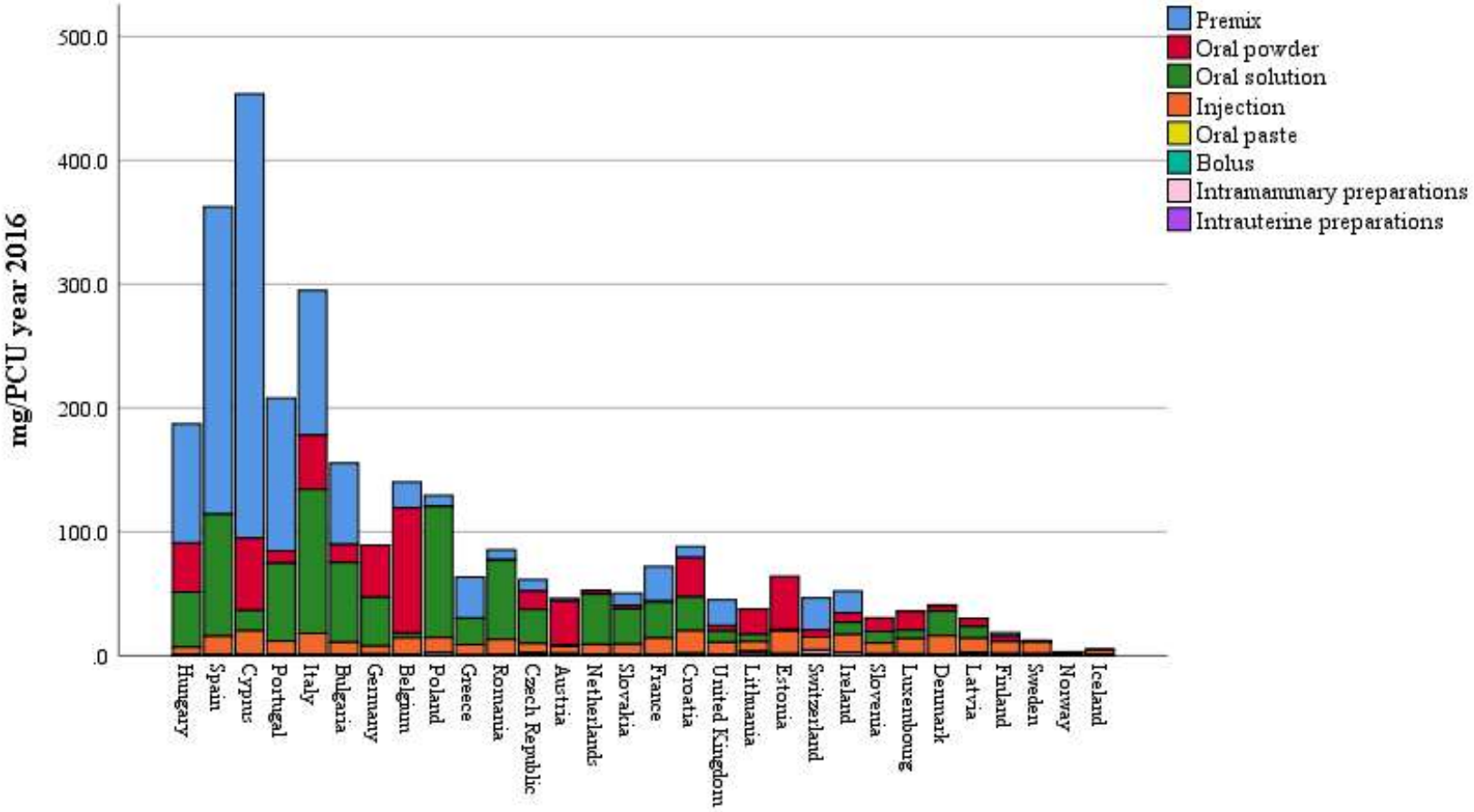


Figure 50. Sales in mg/kg PCU by pharmaceutical form, ordered by the percentage of oral forms for group treatment, year 2016.

The most sold pharmaceutical form in mg/kg PCU is premixes. From those Cyprus is the country that sells the most (358.7 mg/kg PCU), followed by Spain (248.2). Eleven countries (Denmark, Estonia, Germany, Iceland, Latvia, Lithuania, Luxembourg, Netherlands, Norway, Slovenia and Sweden) have sales of premixes below 1 mg/kg PCU.

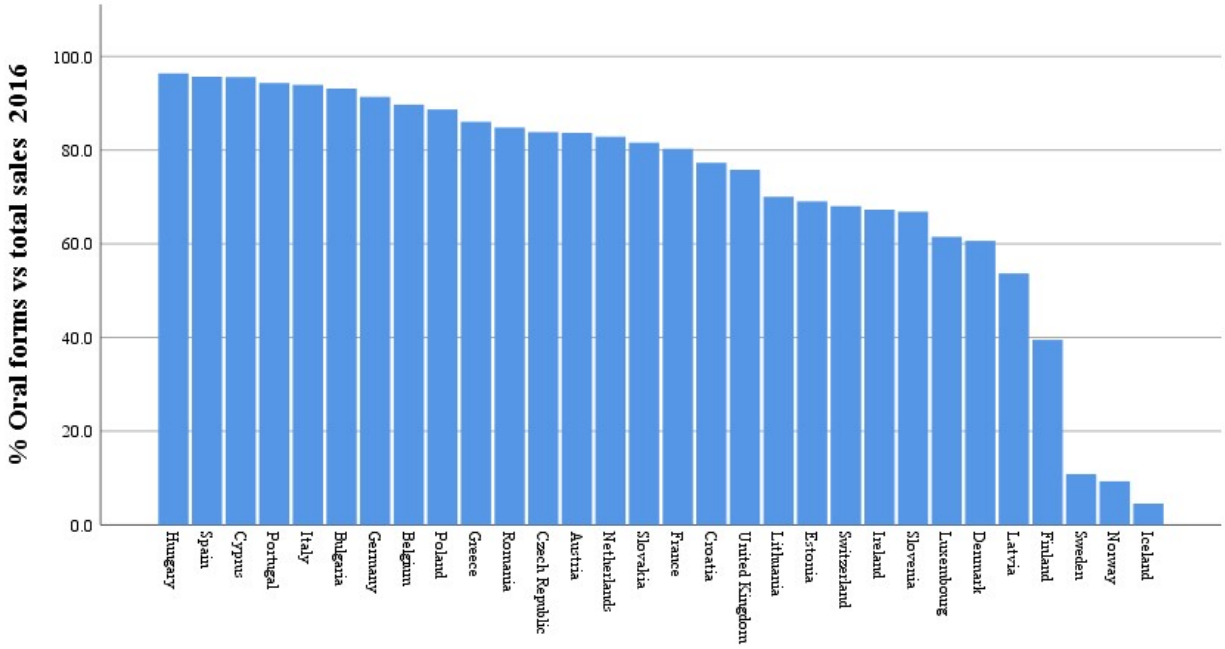


Figure 51. Percentage of oral forms for group treatment *vs* total sales in 2016.

Seven countries have percentages of sales for group treatment that are between 90 and 100% (Hungary, Spain, Cyprus, Portugal, Italy, Bulgaria and Germany), nine for which the percentage is between 80 and 90% (Belgium, Poland, Greece, Romania, Czech Republic, Austria, the Netherlands, Slovakia and France). The countries with the lowest sales in the percentage of oral forms for group treatment are Sweden (10.8%), Norway (9.3%) and Iceland (4.5%).

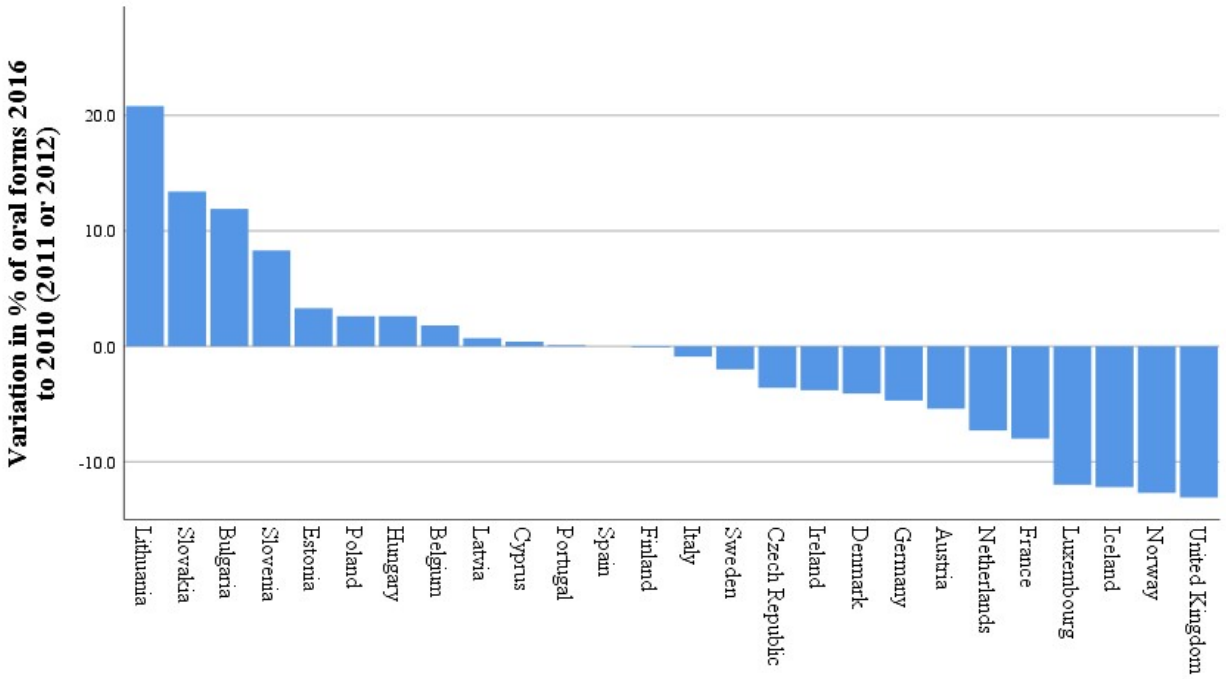


Figure 52. Variation in % of oral forms for group treatment from the years 2016 to 2010 (2011 or 2012).

The biggest increase in the % of oral forms for group treatment use between 2010 and 2016 is in Lithuania (20.8%), whilst the biggest decrease is in the United Kingdom (-13.1%).

The variation in % of oral forms from the years 2016 to 2010 (2011 or 2012) is not statistically different from zero (p = 0.570, 2-tailed).

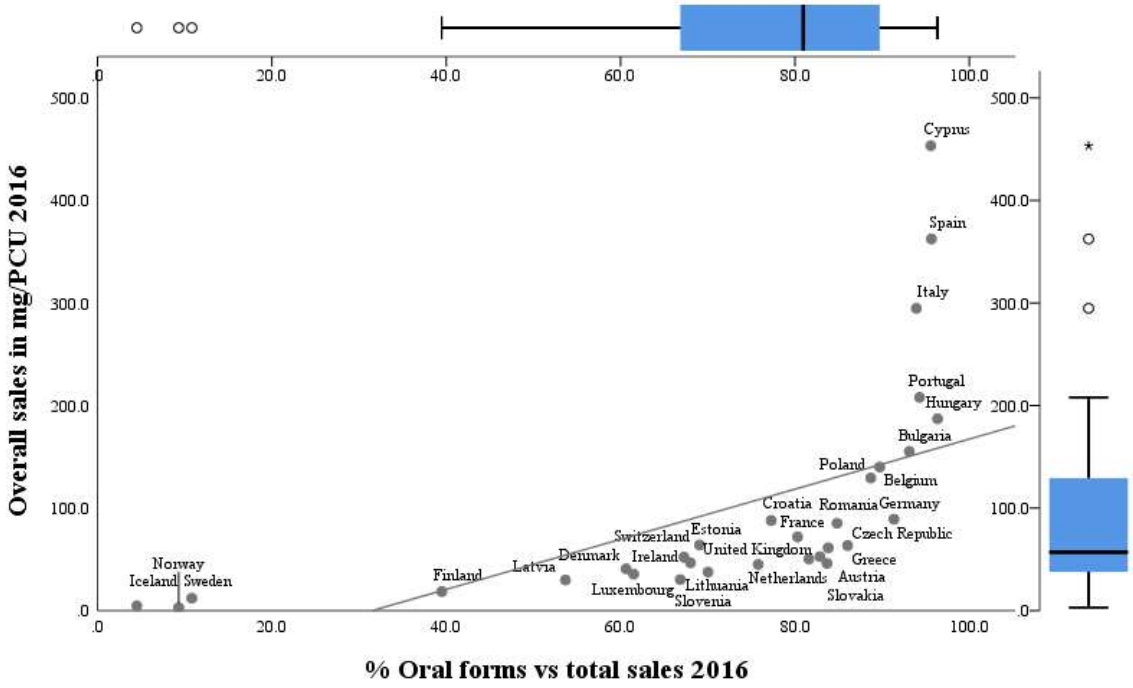


Figure 53. Sales in mg/kg PCU in 2016 *vs* percentage of oral forms for group treatment in 2016.

The Pearson's linear correlation of the sales in mg/kg PCU in 2016 *vs* percentage of oral forms for group treatment in 2016 is moderate (0.585) and significant at the cut-off point of the Bonferroni correction (<0.00147 level, 2-tailed).

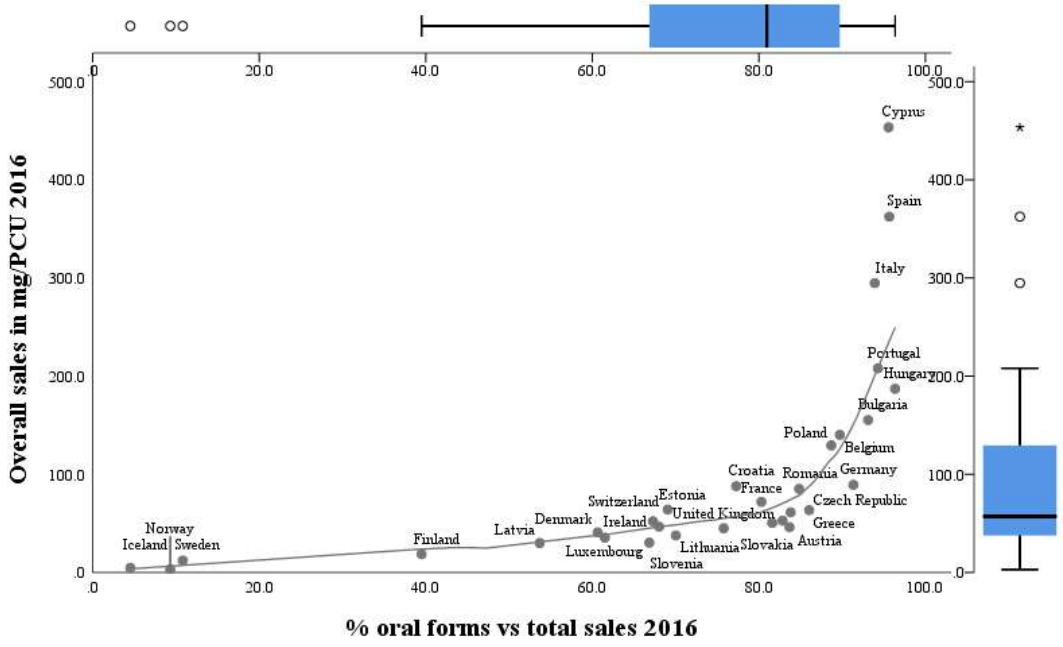


Figure 54. Sales in mg/kg PCU in 2016 *vs* percentage of oral forms for group treatment in 2016, **with a non-linear fit (Loess fit).**

The exponential fit of the overall sales in mg/kg PCU in 2016 *vs* the percentage of oral forms for group treatment in 2016 was computed.

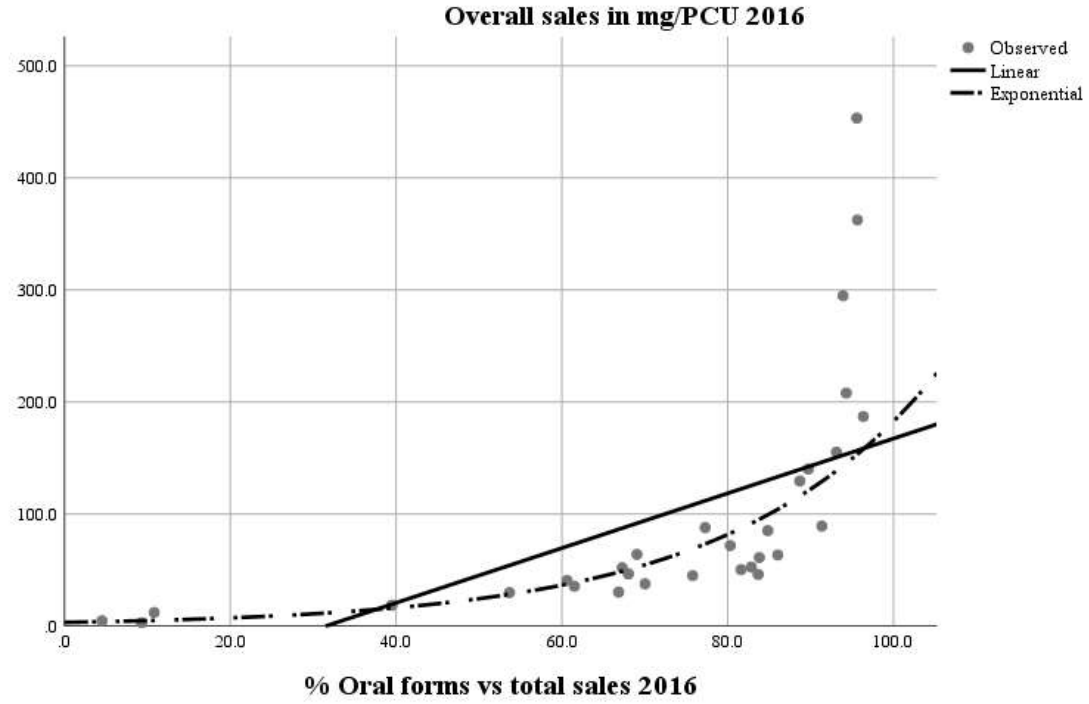


Figure 55. Sales in mg/kg PCU in 2016 *vs* percentage of oral forms for group treatment in 2016, with **exponential and linear fit**.

The coefficient of determination, r squared or r^2, is the proportion of the variance in the dependent variable (overall sales in mg/kg PCU 2016) that is predictable from an independent variable (% oral forms *vs* total sales 2016) and a statistical measure of how close the data are fitted in a regression line. The adjustment using a linear regression gives an r^2 of 0.342, significant at the 0.001 level, while the r^2 for an exponential fit is 0.835, significant with p = 1.9E-12.

The adjustment line shows an increase in mg/kg PCU as the percentage of oral group treatment increases. As the graph shows, an exponential relationship (0.835) between both indicators fits better than a linear one (0.342).

9.3.1. Variation in the percentage of oral forms for group treatment *vs* variation of total sales

The variation in the percentage of oral forms for group treatment through the years 2016 to 2010 (2011 or 2012) - for Bulgaria, Cyprus, Germany, Lithuania, Netherlands, Poland, Slovakia and Spain, the data are from the year 2011, and for Luxembourg for the year 2012 - was analysed in order to identify if an increase or decrease in the percentage of oral sales was correlated to the increase or decrease in total sales in mg/kg PCU for the same years.

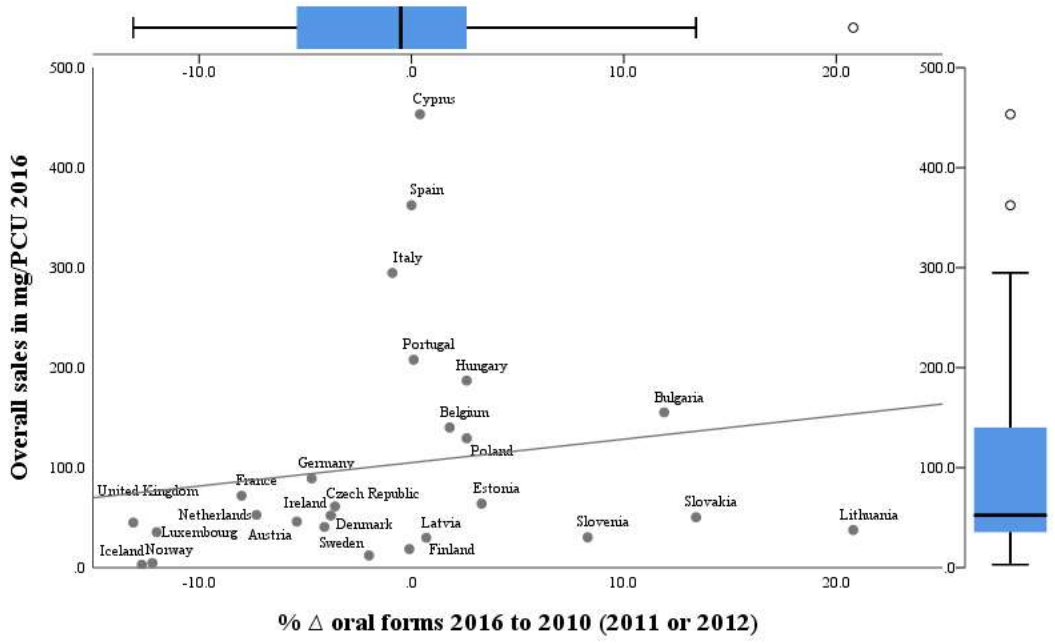


Figure 56. Overall sales in mg/kg PCU in 2016 *vs* the variation in the percentage of the oral forms between the years 2016 to 2010 (2011 or 2012).

The Person's linear correlation of the overall sales in mg/kg PCU in 2016 *vs* the variation in the percentage of the oral forms between the years 2016 to 2010 (2011 or 2012) is not significant ($p = 0.413$, 2-tailed).

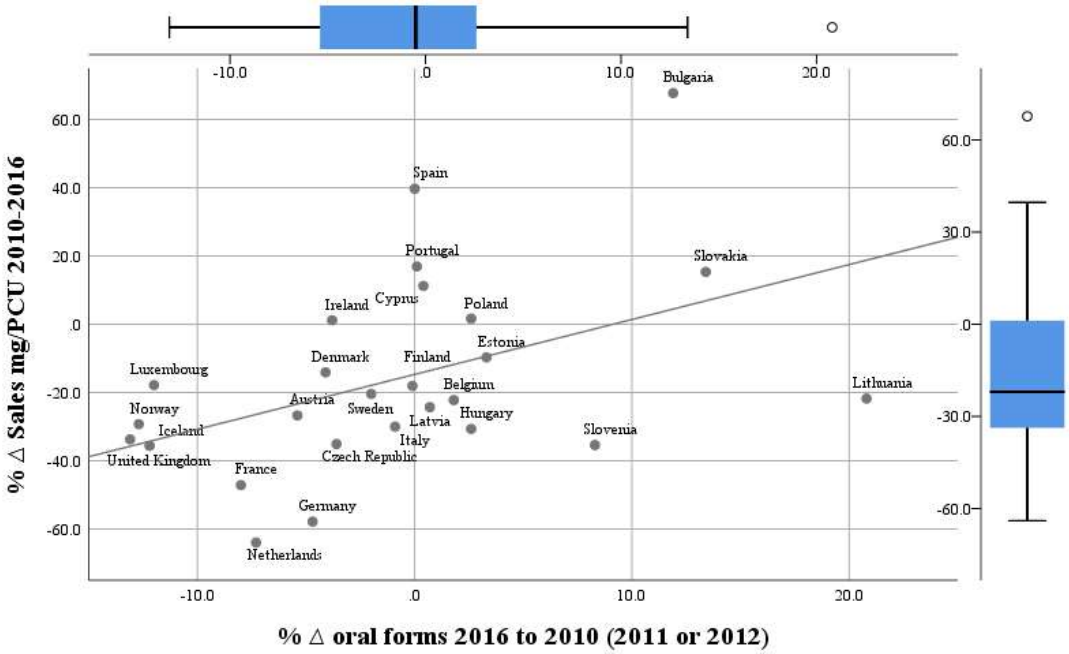


Figure 57. Variation of sales in mg/kg PCU between the years 2016 to 2010 (2011 or 2012) *vs* the variation in oral forms for group treatment during the same years.

The correlation of the variation of sales in mg/kg PCU between the years 2016 to 2010 (2011 or 2012) *vs* the variation in oral forms for group treatment during the same years is not significant ($p = 0.384$, 2-tailed).

9.3.1. Sales of oral forms for group treatment in tonnes for the years 2010 to 2016

Table 16. Sales of oral forms (premix, oral-powder and oral solutions) in tonnes for the years 2010 to 2016[§].

Country	Sales oral forms (sum of premix, oral-powder and oral solutions) in tonnes of antimicrobials						
	2010	2011	2012	2013	2014	2015	2016
Austria	55.7	46.3	46.0	47.9	47.4	41.5	36.9
Belgium	262.9	266.3	246.2	236.1	242.9	234.3	215.7
Bulgaria	NA	30.0	30.6	39.9	28.8	40.3	56.8
Croatia	NA				22.2	21.0	20.5
Cyprus	NA	49.3	42.8	45.7	39.8	45.1	44.3
Czech Republic	62.3	51.8	45.7	48.4	48.3	39.9	36.2
Denmark	77.0	60.8	67.7	70.3	66.4	61.3	59.8
Estonia	5.0	5.0	4.7	6.0	7.2	5.7	5.0
Finland	4.6	4.4	4.4	4.7	4.8	4.2	3.8
France	883.1	774.9	647.6	572.1	645.7	405.0	412.6
Germany	NA	1747.7	1633.0	1456.7	1231.4	784.8	711.8
Greece	NA					66.4	68.7
Hungary	194.5	141.6	171.2	169.6	144.9	170.1	149.9
Iceland	0.1	0.2	0.1	0.1	0.0	0.0	0.0
Ireland	65.1	56.7	65.8	65.5	64.4	68.6	68.8
Italy	1825.6	1577.3	1445.2	1237.2	1242.1	1221.1	1139.4
Latvia	3.4	4.1	4.3	3.7	3.9	4.3	2.9
Lithuania	8.2	6.8	6.9	6.0	8.4	8.2	8.9

Luxembourg	NA	NA	1.6	2.0	1.4	1.1	1.2
Netherlands	424.0	326.8	213.6	195.6	185.5	182.2	150.6
Norway	1.4	1.3	2.2	1.4	0.9	0.6	0.5
Poland	NA	430.7	456.7	509.4	512.3	524.8	505.7
Portugal	171.0	155.3	148.8	171.3	180.5	124.5	198.9
Romania	NA				236.8	221.5	225.1
Slovakia	NA	7.4	7.1	11.5	13.0	10.6	9.9
Slovenia	4.9	6.1	4.6	2.4	3.9	2.7	3.6
Spain	1711.7	2286.8	2027.3	2104.4	2839.8	2907.5	2606.4
Sweden	1.6	1.3	1.1	1.1	0.9	1.0	1.1
Switzerland	49.1	49.1	41.1	38.7	34.0	29.1	25.6
United Kingdom	404.9	293.4	392.4	369.2	379.0	339.2	243.7

[§]NA: Not Available

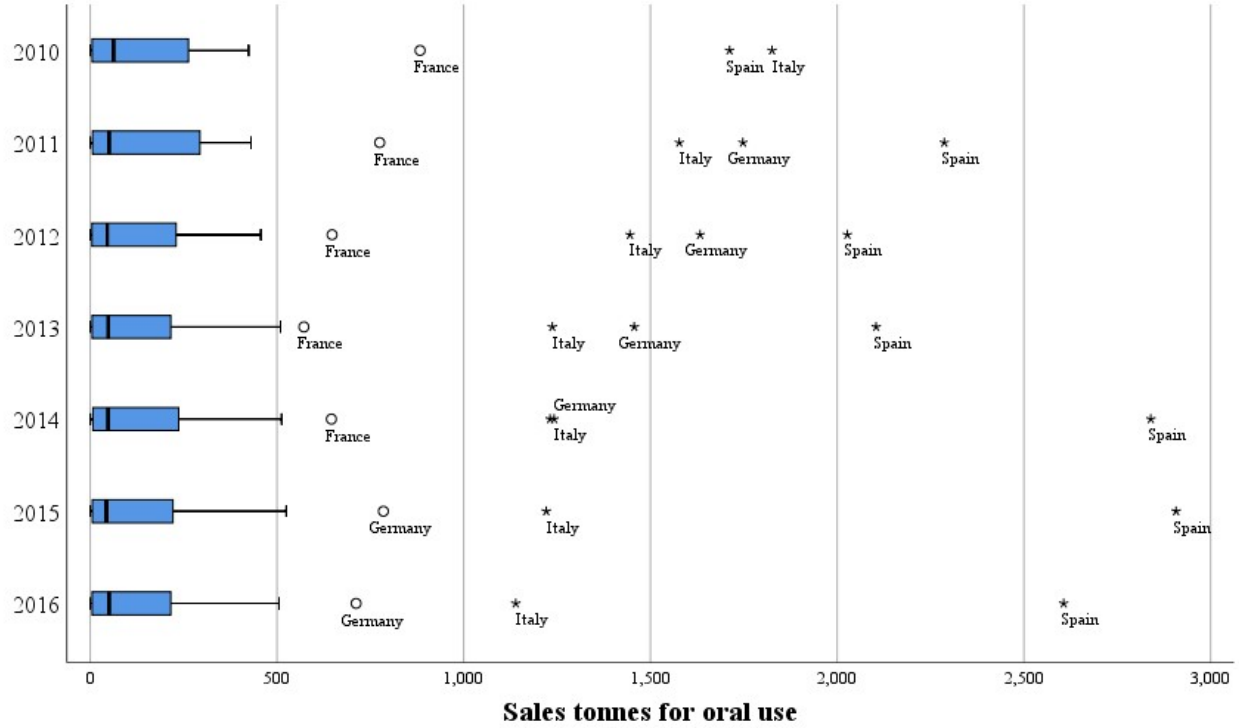


Figure 58. Sales of oral forms for group treatment in tonnes for the years 2010 to 2016. The years in the graph are arranged by placing the 2016 data at the bottom of the graph.

For the years 2010 to 2013 sales in France create a decreasing trend in the total number of tonnes sold.

For the years 2010 to 2016 sales of oral forms also decrease in a remarkable manner for Italy and Germany, in the case of Spain two subsets of data can be observed; between 2010 and 2013 and 2014 to 2016, reflecting the change in data collection in the country.

9.3.2. Results of sales of other pharmaceutical forms

In addition to oral pharmaceutical forms, other pharmaceutical forms were analysed. The injectable, oral paste, bolus, intramammary and intrauterine preparations were grouped under "individual treatments".

Table 17. Distribution of sales, in percentage *vs* the total mg/kg PCU, of veterinary antimicrobial agents applicable for food-producing animals, by administration route/form and country for 2016.

Country	% Premix	% Oral powder	% Oral solution	% Injectables	% Individual treatments	Total mg/kg PCU
Austria	4.3%	***77.2%***	2.2%	12.9%	16.3%	46.1
Belgium	15.0%	72.1%	2.7%	9.8%	10.3%	140.1
Bulgaria	42.3%	9.4%	41.5%	6.4%	6.9%	155.3
Croatia	10.0%	36.3%	31.0%	20.6%	22.7%	87.9
Cyprus	***79.1%***	12.9%	3.6%	4.2%	4.4%	***453.4***
Czech Republic	15.1%	24.0%	44.7%	13.5%	16.2%	61.2
Denmark	1.4%	10.2%	49.1%	37.3%	39.4%	40.8
Estonia	***0.0%***	66.8%	2.2%	28.3%	30.9%	64.
Finland	15.7%	23.6%	***0.1%***	52.3%	60.5%	18.6
France	38.8%	1.4%	40.2%	17.9%	19.7%	71.9
Germany	0.1%	47.1%	44.1%	7.0%	8.6%	89.2
Greece	52.5%	***0.0%***	33.6%	13.9%	14.0%	63.5
Hungary	51.4%	21.2%	23.7%	***3.4%***	***3.6%***	187.1
Iceland	0.2%	2.1%	2.2%	***85.3%***	***95.5%***	4.7
Ireland	33.7%	14.8%	18.8%	27.3%	32.7%	52.1
Italy	39.6%	14.8%	39.5%	5.8%	6.1%	294.8
Latvia	***0.0%***	21.4%	32.2%	37.4%	46.3%	29.9
Lithuania	0.5%	53.9%	15.6%	20.4%	30.0%	37.7
Luxembourg	***0.0%***	42.1%	19.4%	34.4%	38.5%	35.5
Netherlands	0.7%	5.1%	77.0%	15.3%	17.1%	52.7
Norway	3.7%	1.8%	3.8%	61.1%	90.7%	***2.9***

Country	% Premix	% Oral powder	% Oral solution	% Injectables	% Individual treatments	Total mg/kg PCU
Poland	6.5%	0.4%	***81.8%***	9.2%	11.3%	129.4
Portugal	59.3%	4.9%	30.1%	5.5%	5.7%	208.0
Romania	9.2%	0.7%	75.0%	14.9%	15.2%	85.2
Slovakia	19.7%	5.1%	56.8%	16.9%	18.4%	50.4
Slovenia	1.0%	35.3%	30.5%	28.6%	33.2%	30.3
Spain	68.5%	***0.0%***	27.2%	4.3%	4.3%	362.5
Sweden	2.4%	0.6%	7.8%	75.8%	89.2%	12.1
Switzerland	55.9%	12.0%	***0.1%***	22.2%	32.0%	46.6
United Kingdom	45.9%	9.9%	20.0%	22.3%	24.2%	45.0

Blue, bold and italics highlight the lower result or percentage value per column. Red, bold and italics the highest result or percentage per column.

In the above table, the percentage of premix, oral powders, oral solution and individual treatments should add up to 100%. To obtain the total sales, the column corresponding to the percentage of injectables should be excluded, since it is already included in the percentage of individual treatments.

Table 18. Percentage of countries with the lowest and the highest percentage of sales for oral group and individual treatment, and overall sales in mg/kg PCU in 2016.

	Countries	Sales of oral forms for group treatment in %	Treatment of forms for individual treatment	Total sales in mg/kg PCU in 2016
Countries with the lowest percentage of sales for oral group treatment	Iceland	4.5%	95.5%	4.7
	Norway	9.3%	90.7%	2.9
	Sweden	10.8%	89.2%	12.1
Countries with the highest percentage of sales for oral group treatment	Hungary	96.4%	3.6%	187.1
	Spain	95.7%	4.3%	362.5
	Cyprus	95.6%	4.4%	453.4
	Portugal	94.3%	5.7%	208.0
	Italy	93.9%	6.1%	294.8

The table above shows how the countries with the lowest percentage of sales for oral group treatment (and some of highest use of pharmaceutical forms for individual treatment) have low overall AMC and vice versa, showing that there is a clear correspondence between the overall sales of antimicrobials and the sales of oral forms. As mentioned before, the Pearson's linear correlation of the sales in mg/kg PCU in 2016 *vs* percentage of oral forms for group treatment in 2016 is moderate (0.585) and significant with the Bonferroni correction.

9.3.2.1. Results of sales of premixes

The percentage of the sales of premixes *vs* the total sales of antimicrobials in 2016 was analysed.

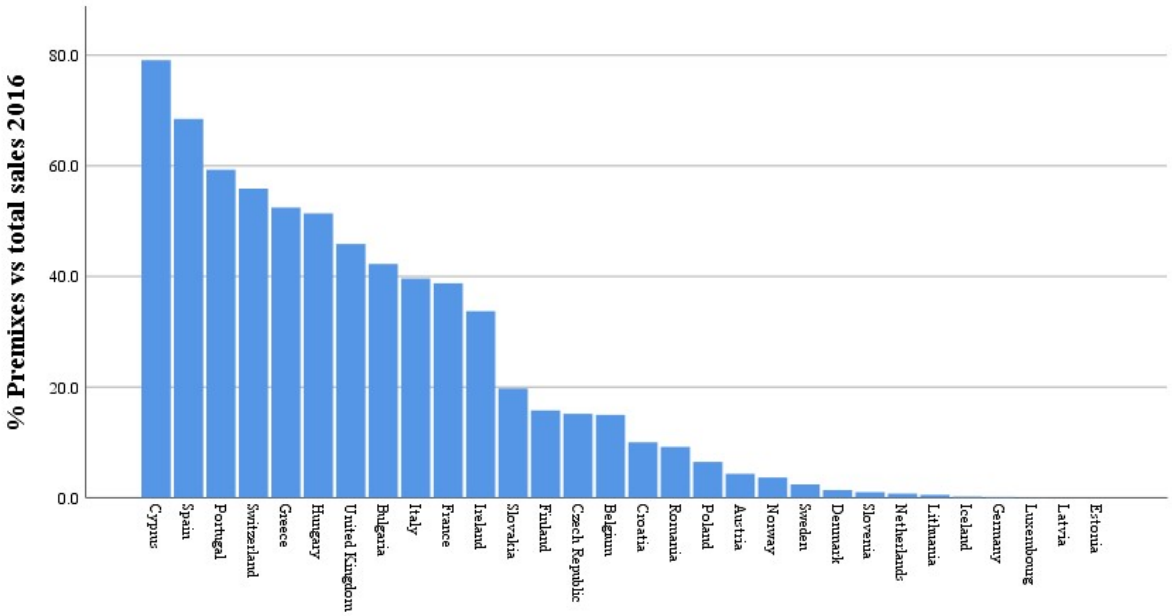


Figure 59. Percentage of premixes *vs* total sales in 2016.

For two countries the percentage of premixes are above 65%; Cyprus (79.1%) and Spain (68.5%). For 8 countries the percentage of sales of premixes are equal or below 1% (Slovenia, Netherlands, Lithuania, Iceland, Germany, Luxembourg, Latvia and Estonia,).

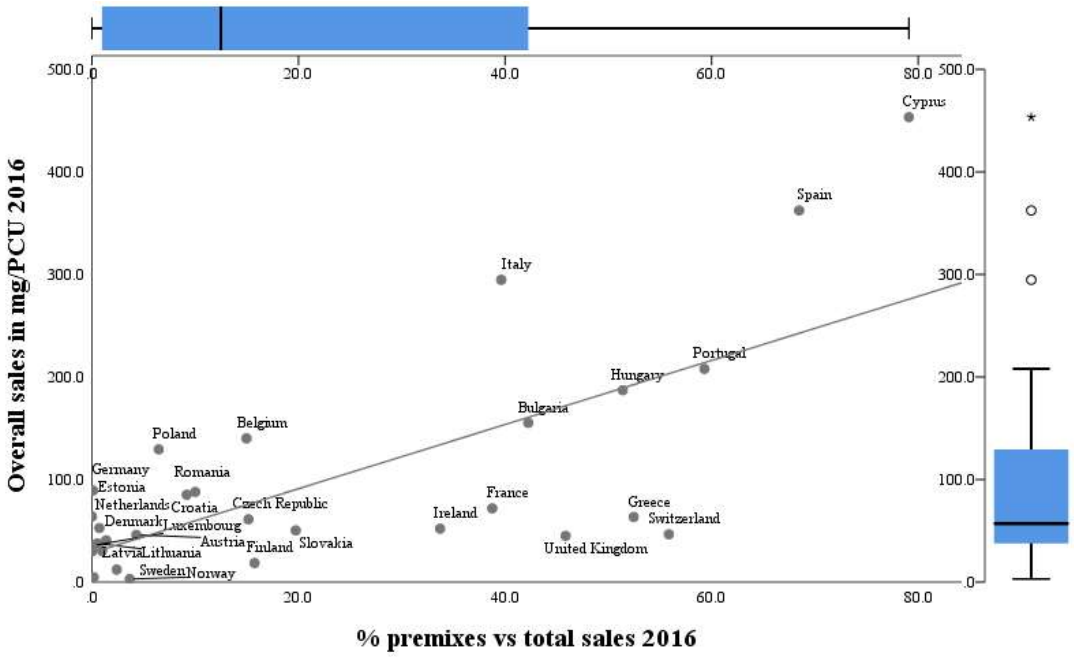


Figure 60. Overall sale in antimicrobials in mg/kg PCU in 2016 *vs* the percentage of premixes in 2016.

Pearson's linear correlation of the sales in mg/kg PCU in 2016 *vs* percentage of premixes in 2016 is significant at the 0.01 level (2-tailed). The Pearson correlation is strong (0.716) and significant at the cut-off point of the Bonferroni correction.

9.3.2.2. Results of sales of oral powders

The percentage of the sales of oral powders *vs* the total sales of antimicrobials in 2016 was analysed.

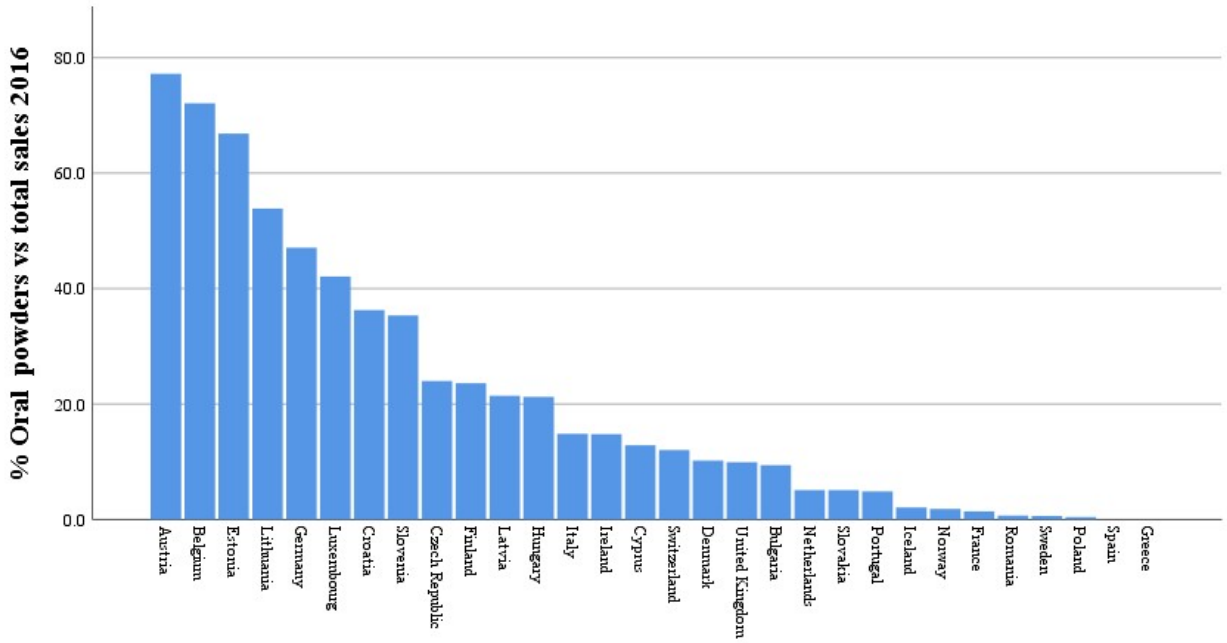


Figure 61. Percentage of oral powders *vs* total sales in 2016.

For three countries (Austria, Belgium and Estonia), sales of antimicrobials as oral powders are above 60%. For five countries the percentage of oral powders is below 1% (Romania, Sweden, Poland, Spain and Greece).

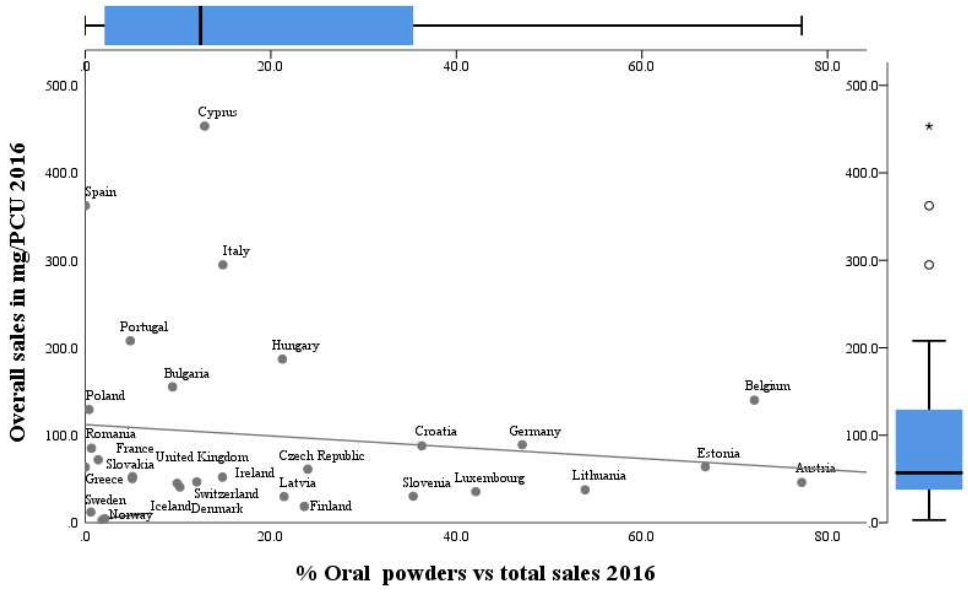


Figure 62. Overall sale in antimicrobials in mg/kg PCU in 2016 *vs* the percentage of oral powders in 2016.

The Pearson's linear correlation of overall sales of antimicrobials in mg/kg PCU in 2016 *vs* the percentage of oral powders is not significant ($p \geq 0.05$, 2-tailed).

9.3.2.3. Results of sales of oral solutions

The percentage of the sales of oral solutions *vs* the total sales of antimicrobials in 2016 was analysed.

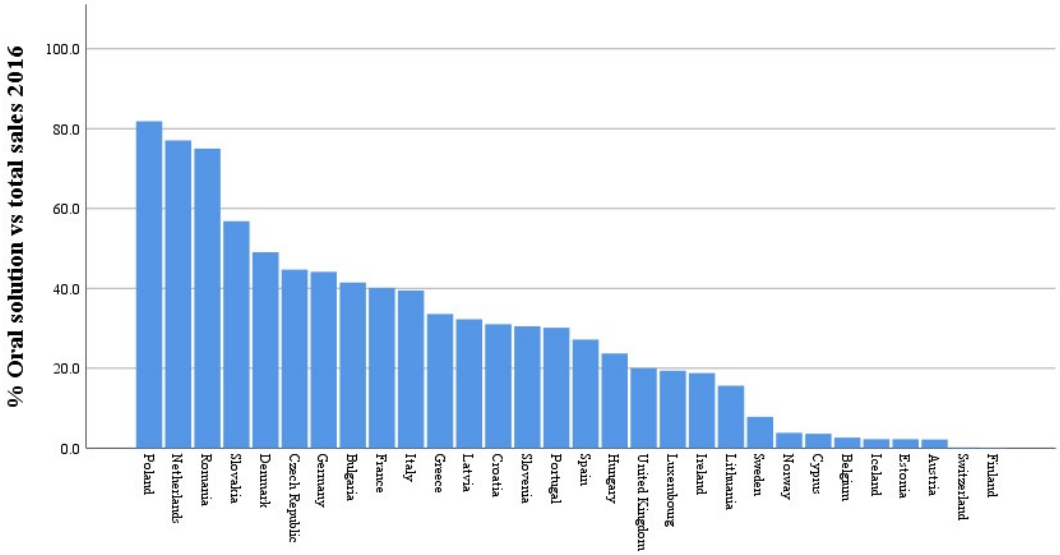


Figure 63. Percentage of oral solutions *vs* total sales in 2016.

For one country, the sales of oral solutions were above 80% (Poland, 81.8%). For two countries (Switzerland and Finland) the sales of oral solutions in 2016 were below1%.

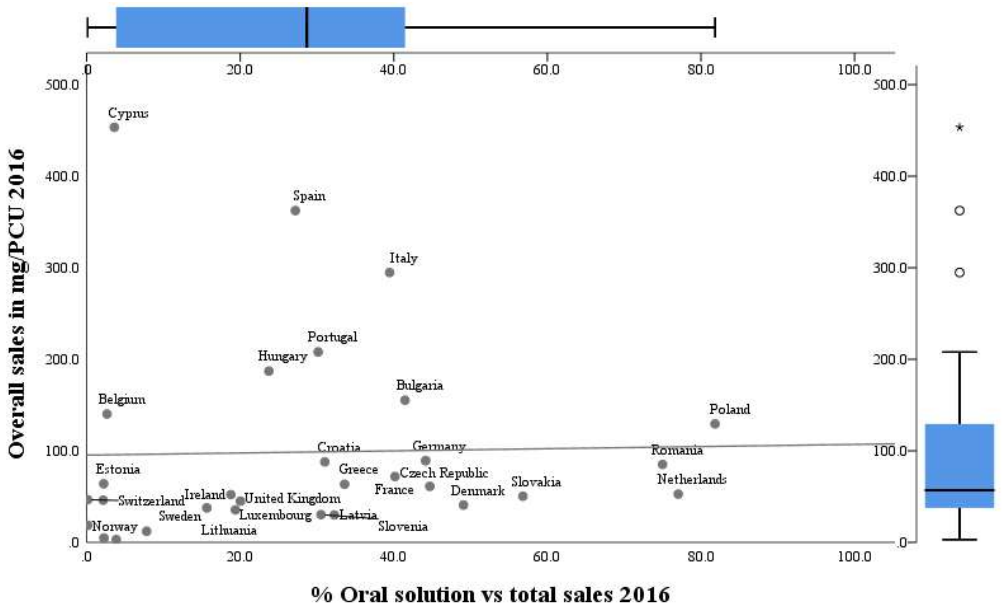


Figure 64. Overall sale in antimicrobials in mg/kg PCU in 2016 *vs* the percentage of oral solutions in 2016.

The Pearson's linear correlation of the sales in mg/kg PCU in 2016 *vs* oral solutions in 2016 is not statistically different from zero at the 0.05 level (2-tailed).

9.3.2.4. Results of sales of injectables

The percentage of the sales of injectables *vs* the total sales of antimicrobials in 2016 was analysed.

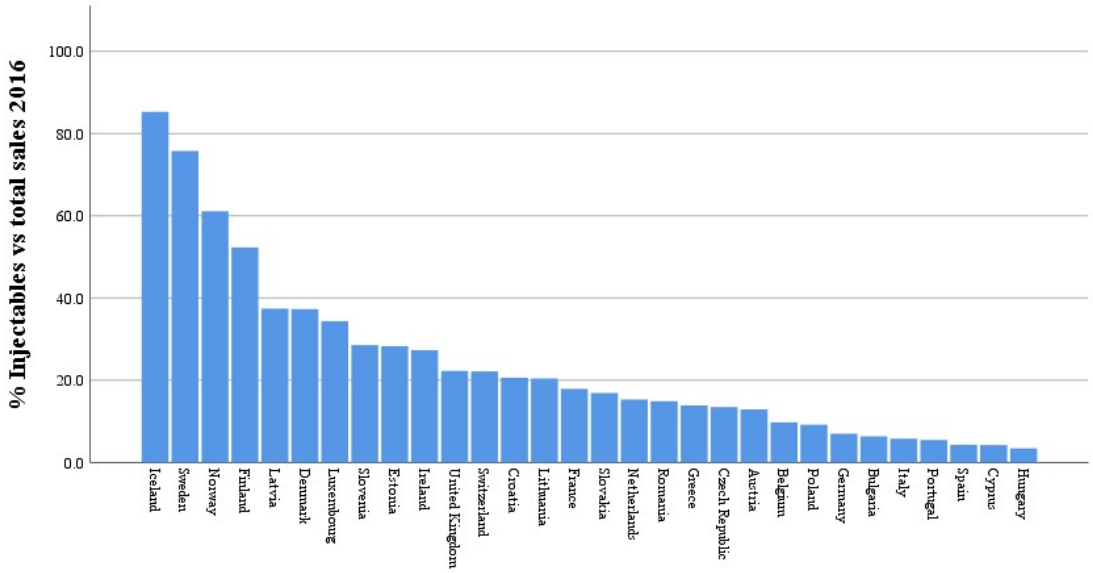


Figure 65. Percentage of injectables *vs* total sales in 2016.

For three countries the sales of injectables are above 60% in 2016: Iceland (85.3%), Sweden (75.8%) and Norway (61.1%). For nine countries the sales of injectables in 2016 were below 10%: Hungary (3.4%), Cyprus (4.2%), Spain (4.3%), Portugal (5.5%), Italy (5.8%), Bulgaria (6.4%), Germany (7.0%), Poland (9.2%) and Belgium (9.8%).

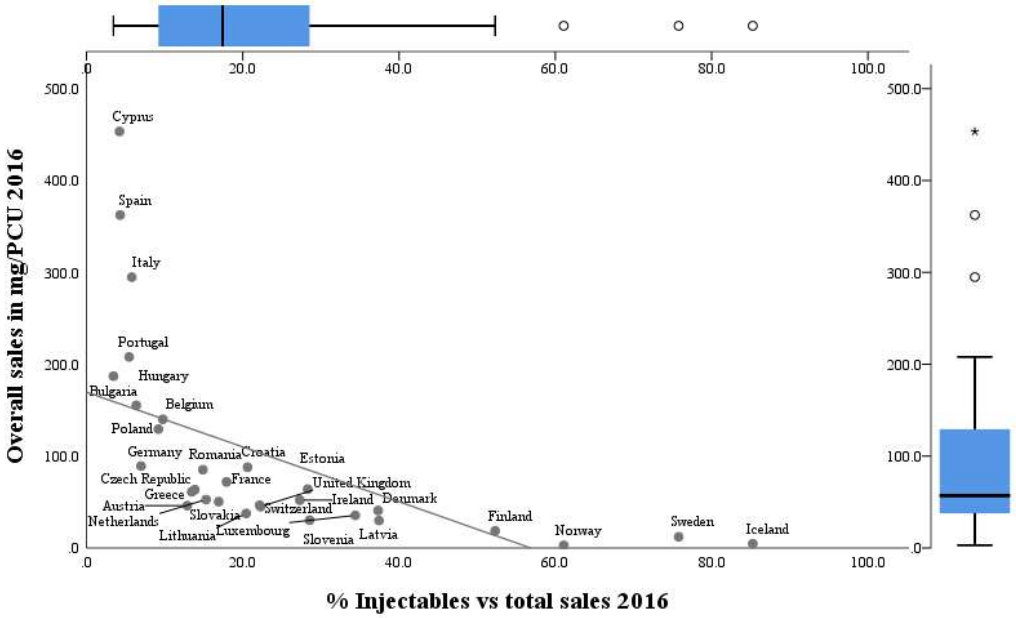


Figure 66. Correlation between the percentage of injectables *vs* total sales in 2016.

Pearson's linear correlation of the sales in mg/kg PCU in 2016 *vs* percentage of injectables in 2016 is significant at the cut-off point of the Bonferroni correction. The correlation is moderate (-0.583).

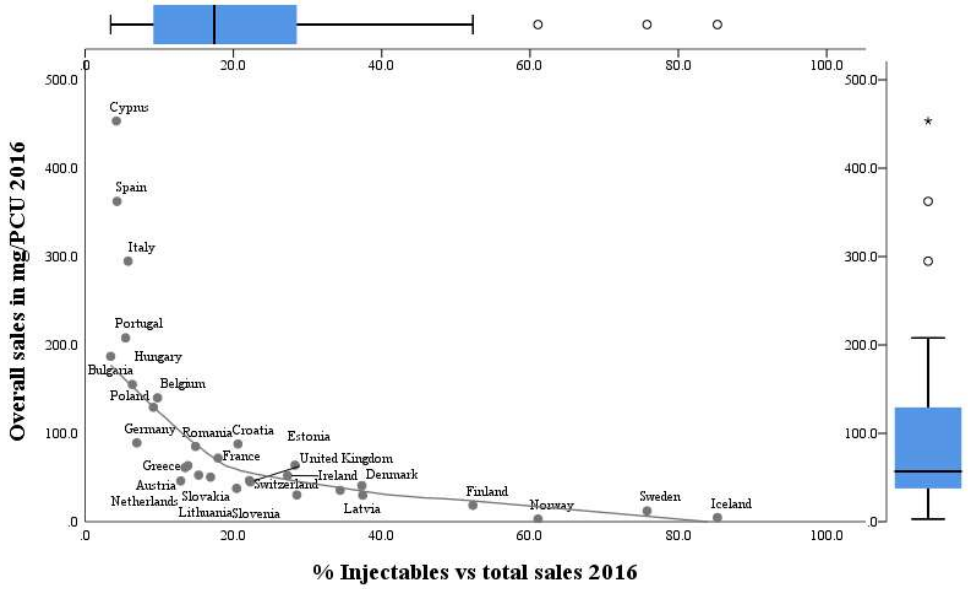


Figure 67. Correlation of the percentage of injectables *vs* total sales in 2016, **with a non-linear fit (Loess fit)**.

The exponential fit of the overall sales in mg/kg PCU in 2016 *vs* the percentage of injectables in 2016 was computed.

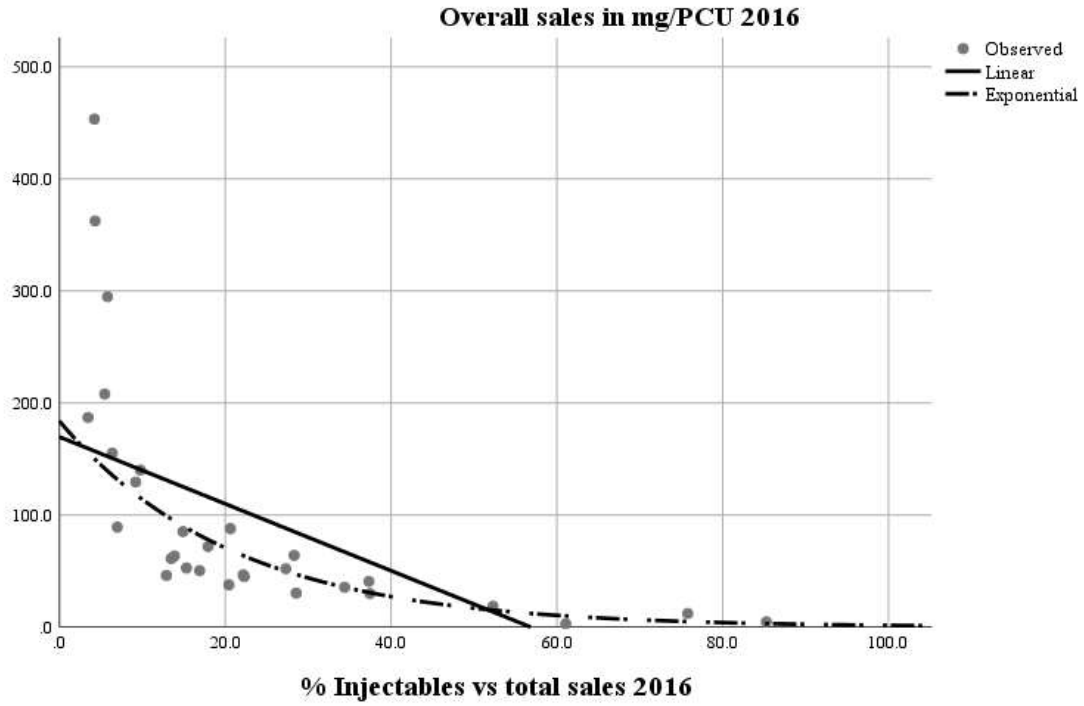


Figure 68. Sales in mg/kg PCU in 2016 *vs* percentage of injectables in 2016, with **exponential and linear fit**.

The adjustment using a linear regression gives an r^2 of 0.339, significant at the 0.001 level, while the r^2 for the exponential fit is 0.784, significant with p = 8.0E-11.

The adjustment line shows a decay in mg/kg PCU as the percentage of injectables increases. As the graph shows, an exponential relationship (0.784) between both indicators fits better than a linear one (0.339).

9.3.2.5. Results of sales of individual treatments

The so-called "individual treatments" was composed of the addition of the percentage of sales of injectables, oral paste, bolus, intramammary and intrauterine preparations.

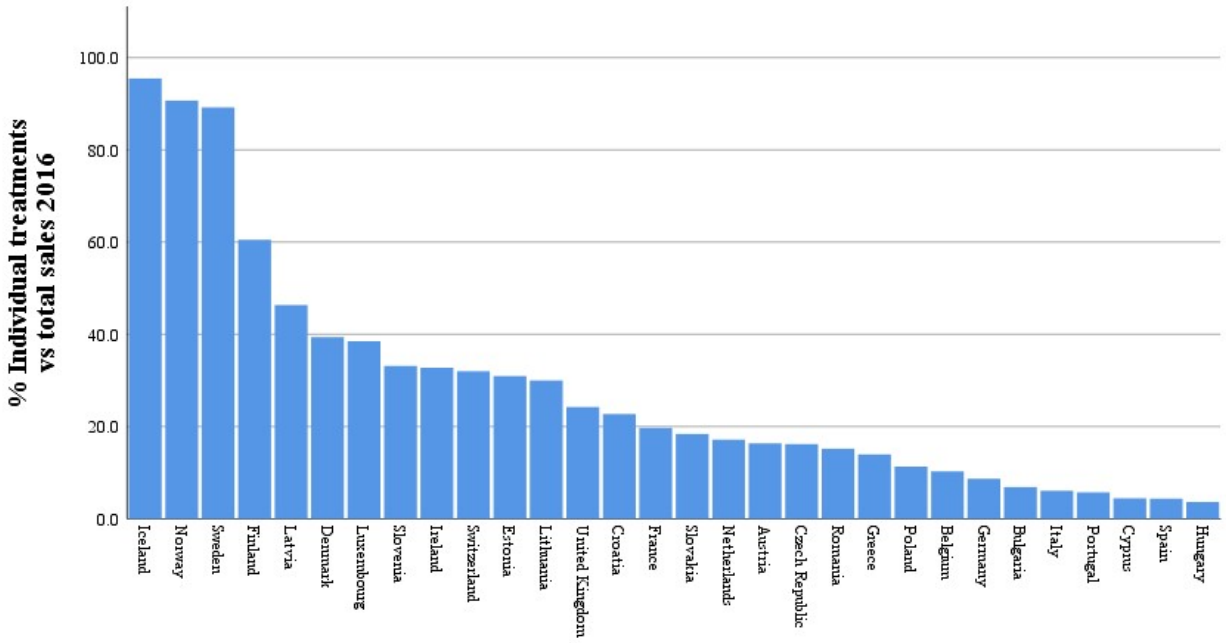


Figure 69. Percentage of individual treatments *vs* total sales in 2016.

For three countries the sales of individual forms were above 80%: Iceland (95.5%), Norway (90.7%) and Sweden (89.2%). For seven countries sales of individual treatments in 2016 were below 10%: Hungary (3.6%), Spain (4.3%), Cyprus (4.4%), Portugal (5.7%), Italy (6.1%), Bulgaria (6.9%) and Germany (8.6%).

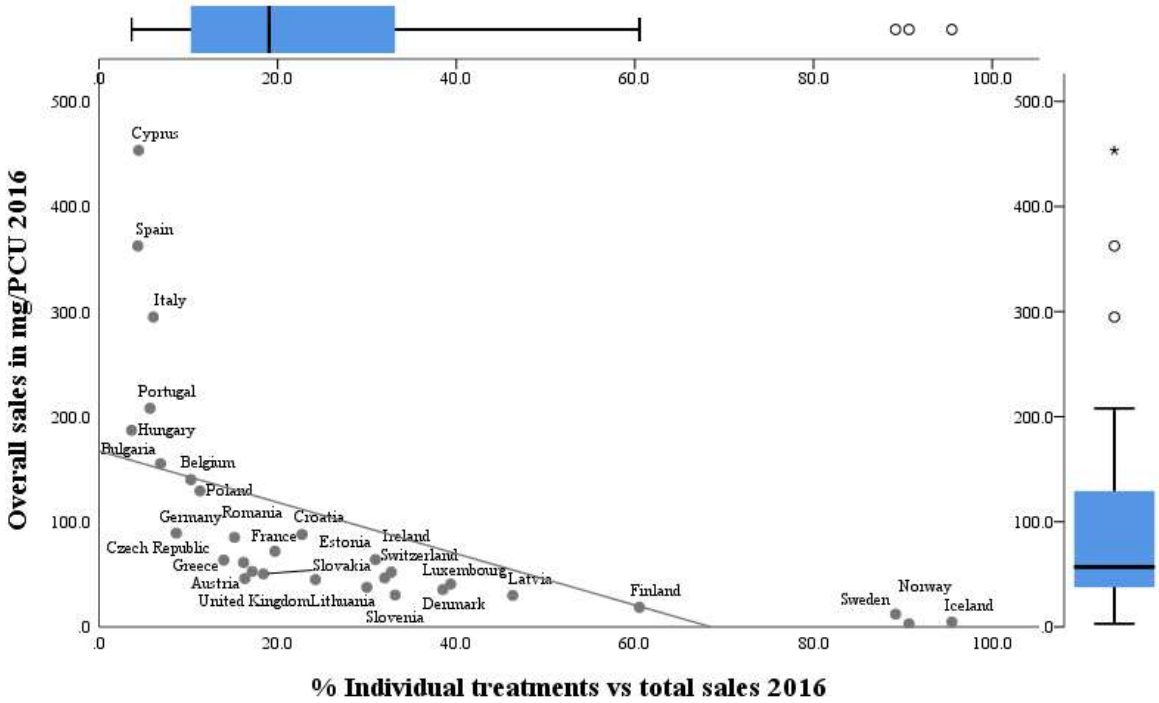


Figure 70. Correlation between the percentage of individual forms *vs* total sales in 2016.

Pearson's linear correlation of the sales in mg/kg PCU in 2016 *vs* percentage of individual treatments in 2016 is significant at the cut-off point of the Bonferroni correction (2-tailed). The correlation is moderate (-0.585).

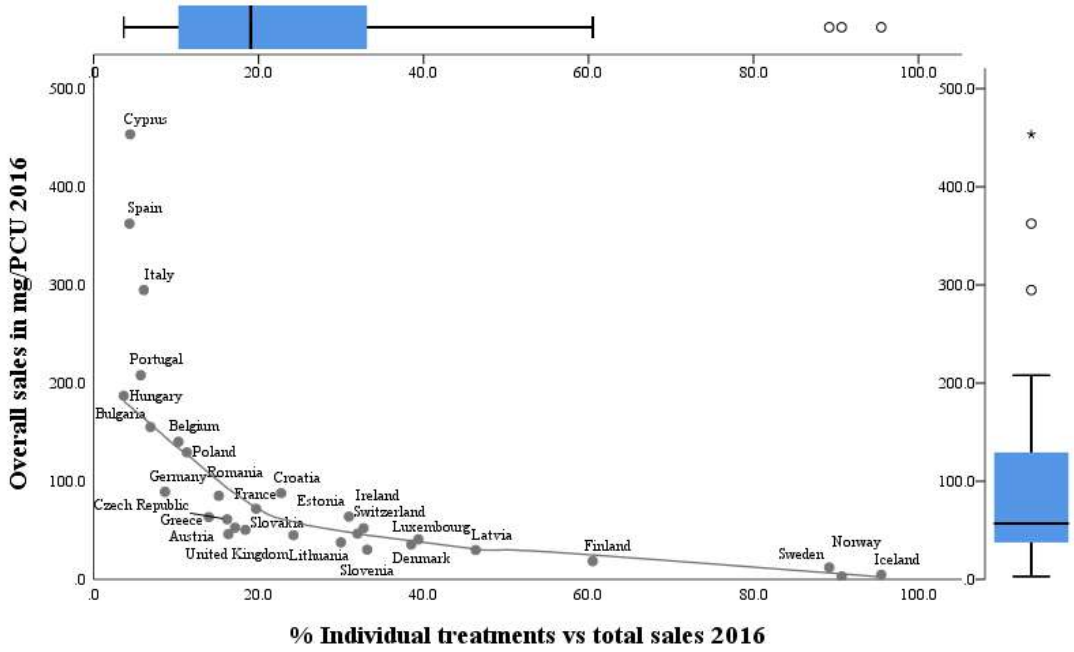


Figure 71. Correlation between the percentage of individual forms *vs* total sales in 2016, **with a non-linear fit (Loess fit).**

The exponential fit of the overall sales in mg/kg PCU in 2016 *vs* the percentage of individual forms in 2016 was computed.

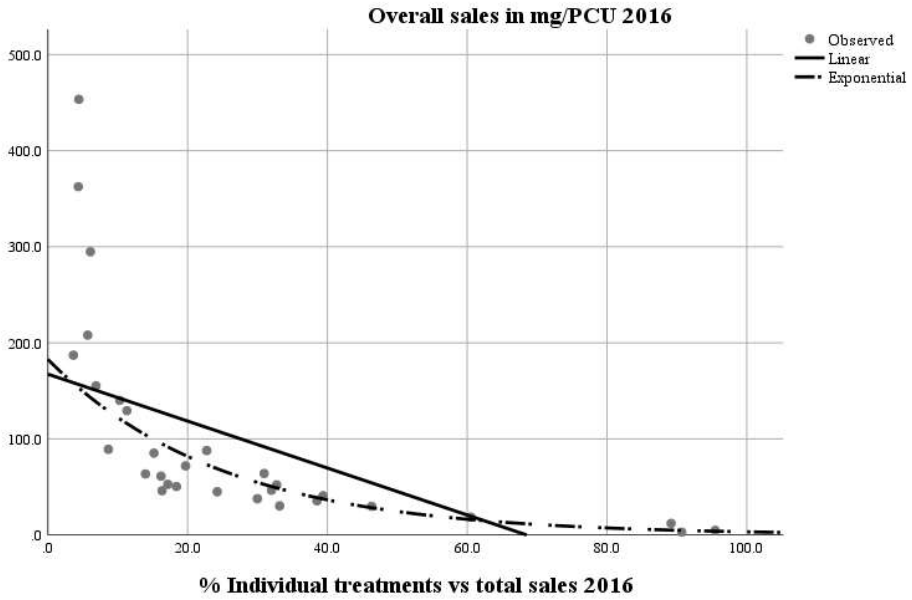


Figure 72. Sales in mg/kg PCU in 2016 *vs* percentage of individual treatments in 2016, with **exponential and linear fit**.

The adjustment using a linear regression gives an r^2 of 0.342, significant at the 0.001 level, while the r^2 for the exponential fit is 0.835, significant with p = 1.9E-12.

As in the previous case, the adjustment line shows a decay in mg/kg PCU as the percentage of individual treatment increases. As the graph shows, an exponential relationship (0.835) between both indicators fits better than a linear one (0.342).

9.3.2.6. Injectable vs individual treatments

When comparing the injectable *vs* the individual treatments, it was evident that sales of antimicrobials of both groups were very similar which can be attributed to sales of the pharmaceutical forms oral paste, bolus, intramammary and intrauterine preparations are small when compared with injectables as shown on the graph below.

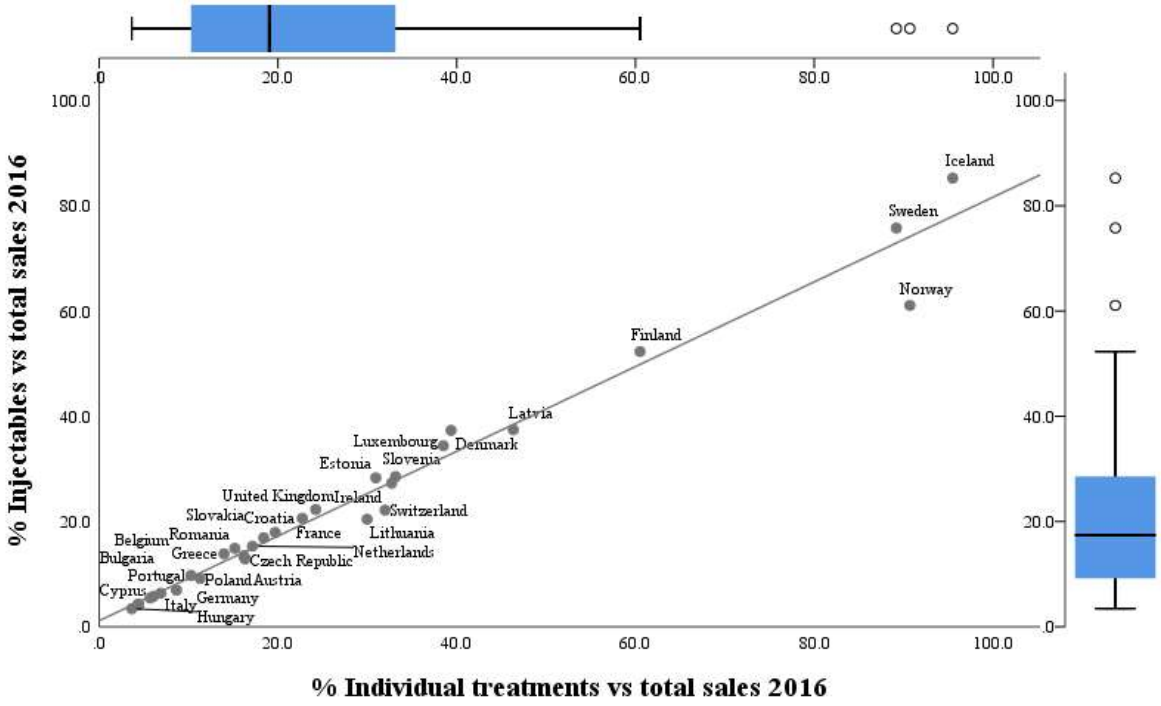


Figure 73. Percentage of injectables *vs* percentage of individual treatments in 2016.

The Pearson's linear correlation of the sales of injectable *vs* individual treatments in 2016 is very strong (0.987) and significant at the cut-off point of the Bonferroni correction (<0.00147 level, 2- tailed).

9.3.3. Other relevant correlations of pharmaceutical forms

The Pearson's linear correlations detailed below are significant according to the Bonferroni correction (<0.00147 level, 2-tailed) (see 9.8.).

The Pearson's linear correlation of the overall sales in mg/kg PCU in 2010 *vs* the percentage in 2016 of oral forms (0.655), premixes (0.628), injectables (-0.650), individual treatments (-0.655) are strong (see Table 31 or Table 36 for details).

The Pearson's linear correlation of the overall sales in mg/kg PCU in 2010 vs the sales in mg/ kg PCU in 2016 of premixes (0.777), oral powders (0.597) and oral solutions (0.620) are strong (or nearly strong) (see Table 31 or Table 36 for details).

9.4. Collecting data on antimicrobial use per animal species association with an overall decrease of antimicrobials expressed as mg/kg PCU in 2016

The collection of AMC data per animal species and its possible link to the amount of antimicrobial sales in mg/kg PCU was analysed. The reason for such analysis is to identify if the action of collecting data per animal species is linked to a reduction on sales of antimicrobials at the country level.

As it was difficult to establish objective criteria to consider if a country was collecting data on AMC by animal species, the inclusion criteria was to consider that the countries collecting data by animal species where those countries for which the "Network on quantification of veterinary **A**ntimicrobial usage at herd level and **A**nalysis, **C**ommunica**T**ion and benchmark**ING** to improve responsible usage" (AACTING) [88] project had identified (in the year 2016) that the countries were collecting data by animal species. AACTING has also produced guidelines for collection, analysis and reporting of farm-level antimicrobial use [88].

Fifteen European countries were identified as collecting data per animal species. Those were: Austria, Belgium, Czech Republic, Denmark, Finland, France, Germany, Ireland, Italy, the Netherlands, Norway, Spain, Sweden, Switzerland and the United Kingdom.

Those countries might collect data from only some types of animals, so the inclusion criteria does not intend to reflect that those countries collect data from all animal species or a representative sample, but that making efforts to collect data by animal species is correlated with a low AMC or a decrease in the use of antimicrobials through the years.

Table 19. Countries collecting data per animal species in 2016.

	Assigned as collecting data per animal species in 2016			
Country	Yes		No	
	Austria	Italy	Bulgaria	Lithuania
	Belgium	Netherlands	Croatia	Luxembourg
	Czech Republic	Norway	Cyprus	Poland
	Denmark	Spain	Estonia	Portugal
	Finland	Sweden	Greece	Romania
	France	Switzerland	Hungary	Slovakia
	Germany	United Kingdom	Iceland	Slovenia
	Ireland		Latvia	

Table 20. Statistics (N, mean and standard deviation) of the countries collecting data per animal species in 2016, sales in mg/kg PCU in 2016.

Collecting data by animal species	Sales of antimicrobials (mg/ PCU)		
	N	Mean	Std. Deviation
No	15	108.1	113.2
Yes	15	89.1	103.4
Total	30	98.6	107.0

The mean of the sales of antimicrobials (mg/kg PCU) of those countries collecting data by animal species is 89.1, the mean of those not collecting those data by animal species is 108.1.

The comparison of the sales of antimicrobials (mg/kg PCU) depending collecting or not data by animal species, using the Mann-Whitney U test, does not allow concluding differences between them ($p = 0.539$, 2-tailed).

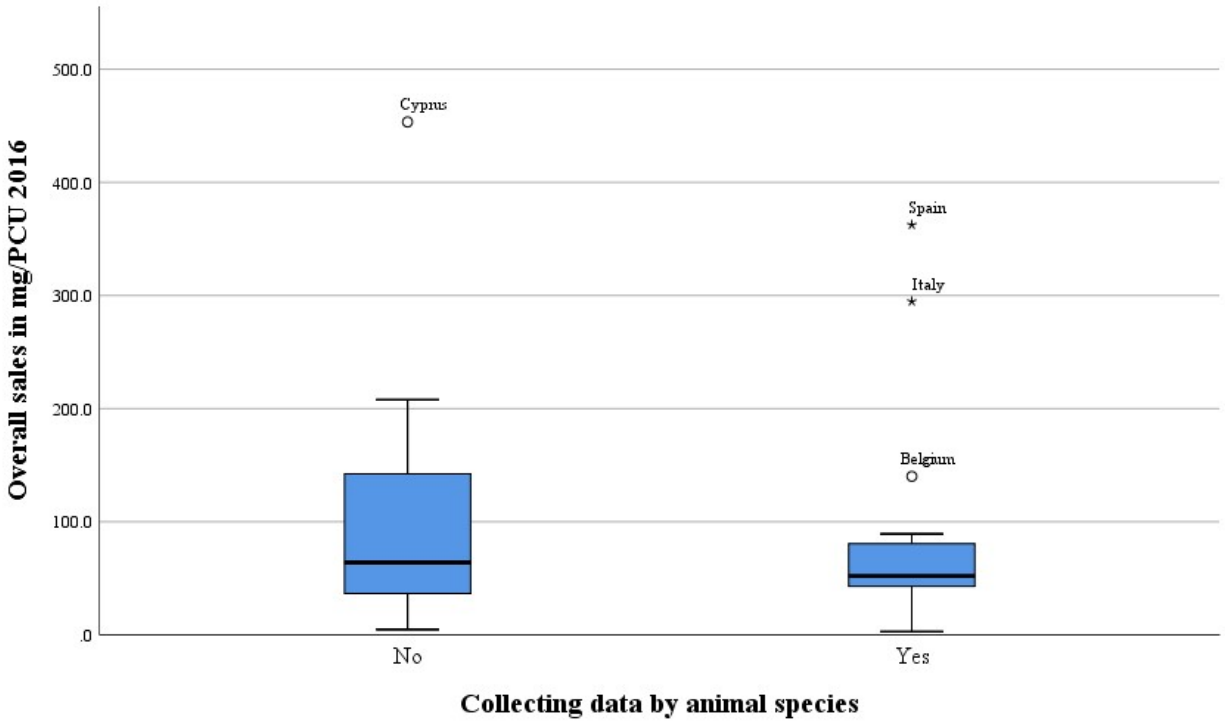


Figure 74. Sales of all antimicrobials in mg/kg PCU in 2016 in countries not collecting data per animal species *vs* those collecting data per animal species.

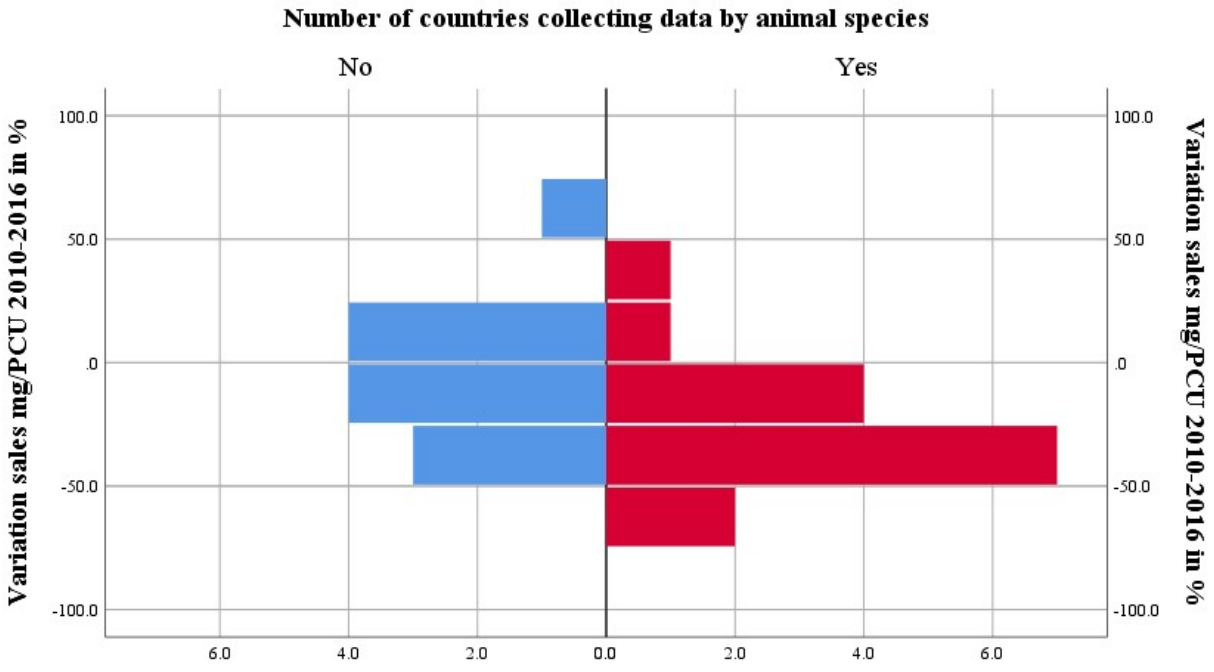


Figure 75. Population pyramid of sales in mg/kg PCU 2016 showing the frequency of countries collection data by animal species.

Figure 76. Map of the collection of data by animal species in 2016.

9.4.1. Variation in mg/kg PCU for the years 2010 to 2016 *vs* collection of data by animal species in 2016

In addition to analysing the sales of antimicrobials in mg/kg PCU for the year 2016, the variation in mg/kg PCU for the years 2010 to 2016 *vs* the collection of data by animal species in 2016 was also investigated.

Table 21. Statistics (N, mean and standard deviation) on the variation in mg/kg PCU for the years 2016 to 2010 (2011 or 2012) *vs* collection of data by animal species in 2016.

Collecting data by animal species	Variation sales of antimicrobials (mg/kg PCU) for the years 2016-2010		
	Number of countries	Mean (in %)	Std. Deviation
No	12*	-5.3	29.9
Yes	15	-26.6	24.8
Total	27	-17.1	28.7

*Data from Croatia, Greece and Romania could not be included for this analysis

In the period 2010 to 2016, the 15 countries collecting data per animal species have a higher reduction of AMC (-26.6%) versus the 12 countries not collecting data by animal species (-5.3%).

The comparison of the variation of AMC (mg/kg PCU) depending collecting or not data by animal species, using the Mann-Whitney U test, does not allow concluding differences between them (p = 0.059, 2-tailed).

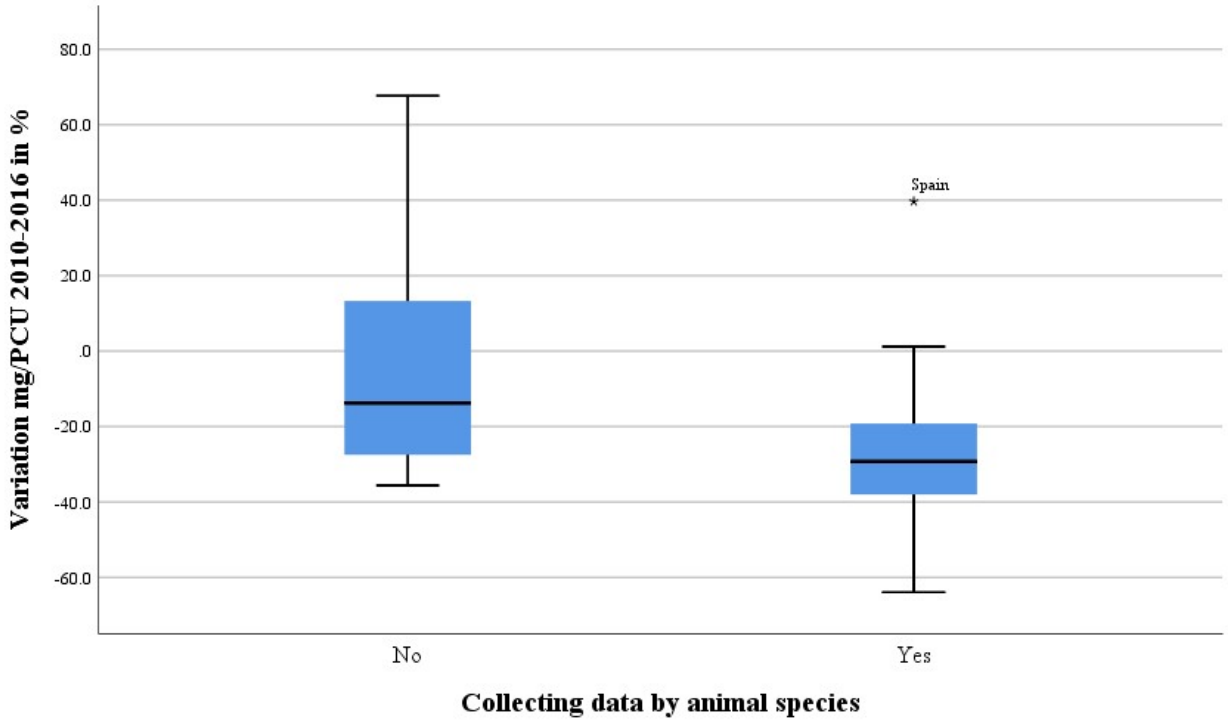


Figure 77. Variation of sales of antimicrobials in % in the period 2010 to 2016, according to the collection of data by animal species.

9.5. Collecting data on antimicrobial sales before 2007 (pre-ESVAC) is associated with low sales of antimicrobials in 2015

As detailed under 8.2.3.7. , the objective of the analysis is to identify if the countries that have been collecting data on AMC (sales or use) for many years have had a significantly lower AMC in the studied period of 2010 (2011 or 2012) to 2016 than those that were not collecting data and if collecting data for a number of years results in a reduction of AMC statistically different from zero between the two groups of countries.

The criteria for deciding which countries were collecting data before the ESVAC project was based on the publication Grave *et al.,* 2010 [195]. Those countries are the Czech Republic, Denmark, Finland, France, Germany, Netherlands, Norway, Sweden, Switzerland and the United Kingdom.

Table 22. Statistics (N, mean and standard deviation) collecting data on antimicrobial sales before 2007 (pre-ESVAC) *vs* the AMC in mg/kg PCU in 2016.

Collecting data on antimicrobial sales before 2007 (pre-ESVAC)	Sales of antimicrobials (mg/kg PCU)		
	N	Mean	Std. Deviation
No	20	125.9	121.5
Yes	10	44.1	27.0
Total	30	98.6	107.0

In the period 2010 to 2016, the 10 countries collecting data on AMC in animals before 2007 have a much lower consumption in mg/kg PCU (44.1) versus the 20 countries not collecting data by 2007 (125.9).

Non-parametric two independent test (Mann-Whitney Test) was applied, concluding that the differences between both groups are significant (p = 0.002, 2-tailed).

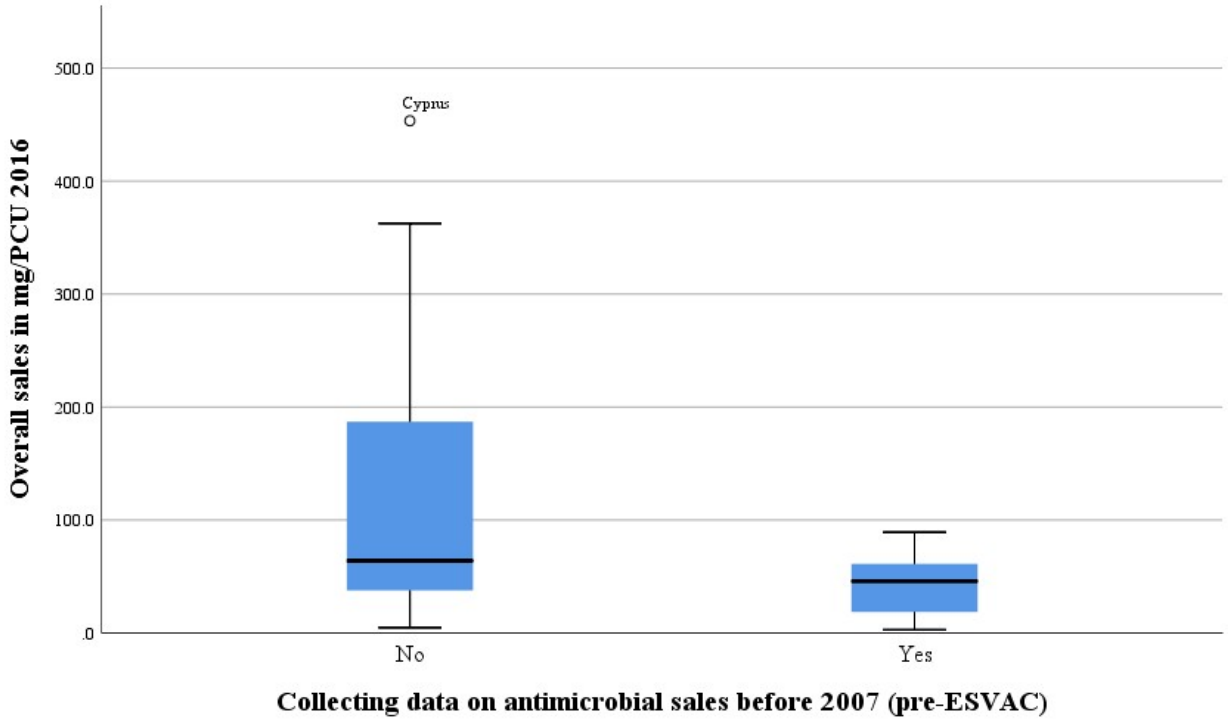


Figure 78. Sales of antimicrobials in mg/kg PCU in 2016 and collecting data on antimicrobial sales before 2007.

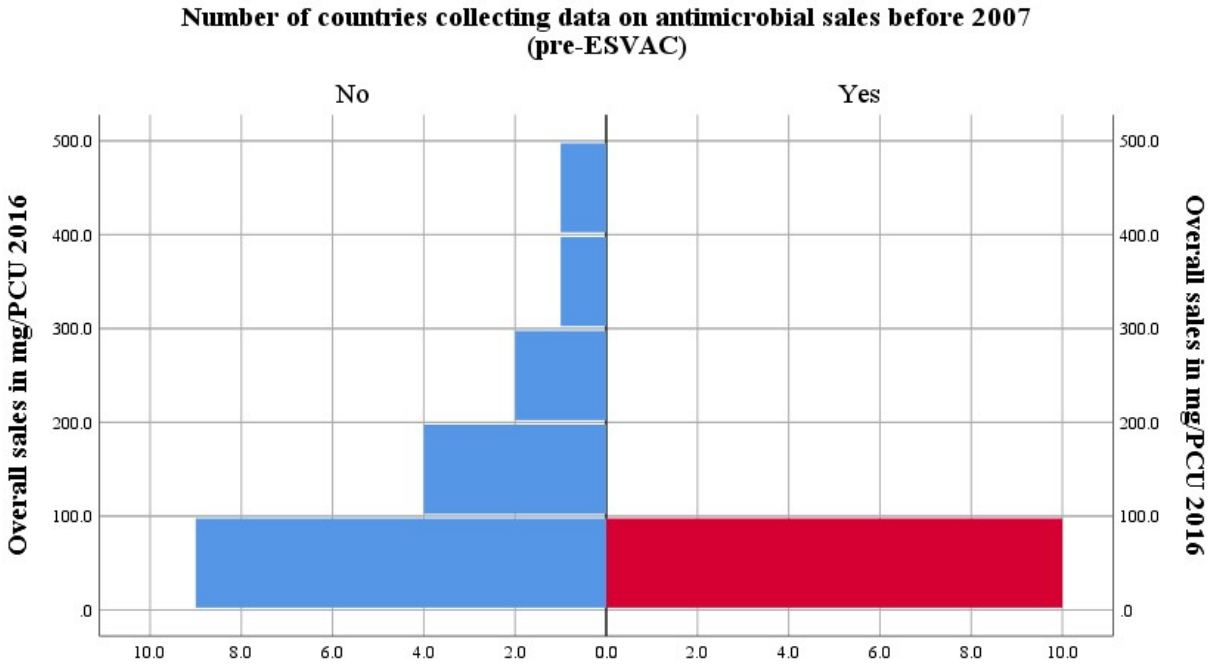


Figure 79. Population pyramid of the sales of antimicrobials in mg/kg PCU in 2016 showing the frequency of countries collecting data on antimicrobial sales before 2007.

Figure 80. ESVAC countries that were collecting data on antimicrobials consumption in animals by 2007.

9.5.1. Variation in the percentage of mg/kg PCU for the years 2010 to 2016 *vs* collecting data on AMC in animals before 2007 (pre-ESVAC)

Table 23. Statistics (N, mean and standard deviation) collecting data on antimicrobial sales before 2007 (pre-ESVAC) *vs* the increase of sales in mg/kg PCU during the years 2010 to 2016.

Collecting data on antimicrobial sales before 2007 (pre-ESVAC)	Variation in the percentage of mg/kg PCU		
	Number of countries	Mean (in %)	Std. Deviation
No	17*	-5.9	28.8
Yes	10	-36.1	16.7
Total	27	-17.1	28.7

*Data from Croatia, Greece and Romania could not be included for this analysis

In the period 2010 to 2016, the 10 countries collecting data on AMC in animals before 2007 have a higher reduction of AMC (-36.1%) versus the 10 countries not collecting data by 2007 (-5.9%).

The Mann-Whitney U test allows us to conclude that the increase in sales in both groups are statistically different (p = 0.006, 2-tailed).

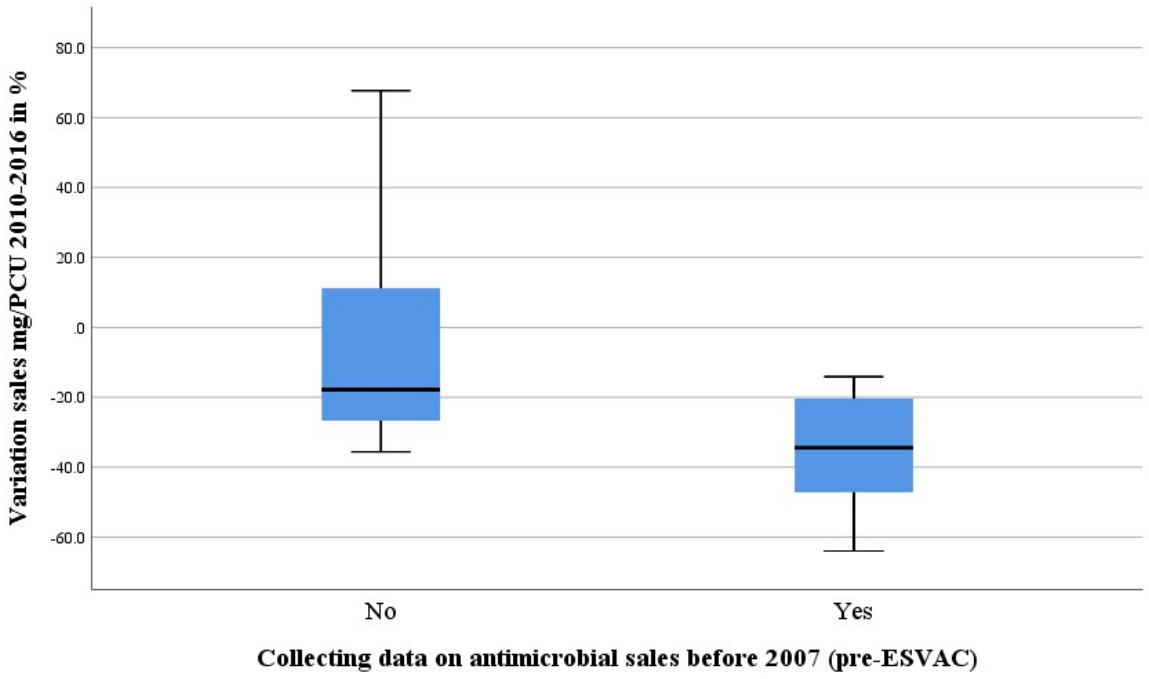


Figure 81. Variation of sales of antimicrobials in percentage in the period 2010 to 2016 if collecting data before 2007 (pre-ESVAC) and those not collecting data.

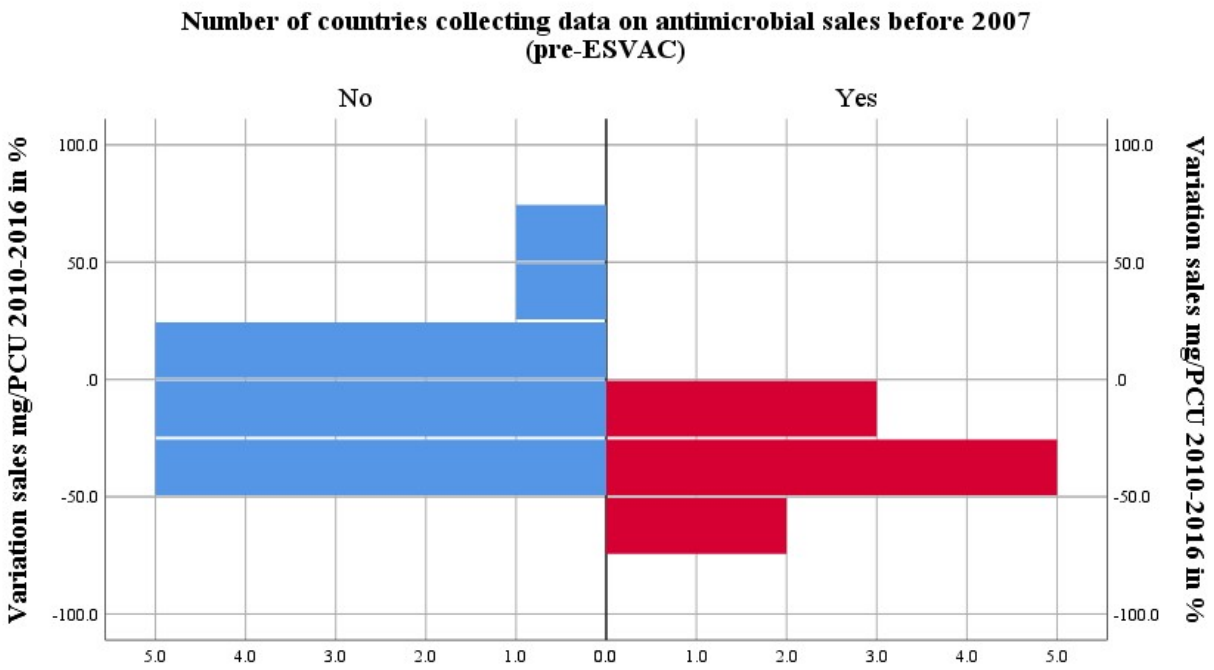


Figure 82. Population pyramid with the variation of sales of antimicrobials in % 2010 to 2016 according to the countries collecting data on sales of antimicrobials before 2007 (pre-ESVAC) and their frequency.

9.6. The animal species produced is associated with the overall sales of antimicrobials

Different animal species will have different requirements for AMC due to the animal species and the production systems.

The animal production by animal species and the consumption of antimicrobials in mg/kg PCU was analysed. For this analysis, the percentage of the Animal Population Correction Unit (PCU in kg) in 2016 was analysed against the mg/kg PCU of antimicrobials.

000 tonnes) of the population of food-producing species for 2016.

igs	Poultry	Sheep & goats	Fish	Rabbits	Horses	Total
369	80	35	0	0	32	957
882	236	16	0	4	121	1,715
83	47	100	0	0	33	393
90	38	47	16	0	0	302
45	13	25	0	0	2	102
205	127	18	21	8	32	705
1,773	123	13	43	0	70	2,420
38	2	7	1	0	4	113
166	73	13	14	0	30	521
1,815	1,145	642	45	47	211	7,143
3,807	1,071	137	19	20	520	8,734
116	128	784	123	0	11	1,258
346	193	97	23	2	21	832

Country	Cattle	Pigs	Poultry	Sheep & goats	Fish	Rabbits	Horses	Total
Italy	1,592	847	755	590	171	29	132	4,116
Latvia	111	37	19	8	0	0	4	180
Lithuania	192	72	56	12	0	0	7	338
Luxembourg	40	12	0	1	0	0	2	55
Netherlands	1,174	1,685	398	94	62	1	33	3,446
Norway	214	130	68	108	1,326	0	50	1,896
Poland	1,547	1,453	1,266	18	0	2	121	4,407
Portugal	228	359	220	174	10	6	18	1,014
Romania	929	553	453	1,001	7	0	173	3,116
Slovakia	93	55	56	31	2	0	4	242
Slovenia	98	19	40	9	2	0	11	178
Spain	918	3,738	834	1,437	308	68	216	7,518
Sweden	298	198	105	48	13	0	142	805
Switzerland	477	203	70	34	0	1	22	806
United Kingdom	1,792	789	1,151	2,845	187	0	378	7,142

Table 25. Calculated percentage of cattle, pigs, poultry, other animal species and pigs and poultry of the population of food-producing species for 2016.

Country	% Cattle	% Pigs	% Poultry	% Pigs + poultry	% Other animal species
Austria	46.1%	38.6%	8.3%	46.9%	7.0%
Belgium	26.5%	51.4%	13.8%	65.2%	8.3%
Bulgaria	33.1%	21.1%	11.9%	33.0%	33.9%
Croatia	36.7%	29.9%	12.4%	42.3%	21.0%
Cyprus	17.0%	44.5%	12.7%	57.2%	25.8%
Czech Republic	41.7%	29.1%	18.0%	47.1%	11.2%
Denmark	16.5%	***73.3%***	5.1%	***78.3%***	5.2%
Estonia	53.6%	33.7%	2.1%	35.8%	10.6%
Finland	43.1%	31.9%	14.1%	45.9%	11.0%
France	45.4%	25.4%	16.0%	41.4%	13.2%
Germany	36.2%	43.6%	12.3%	55.8%	8.0%
Greece	***7.6%***	9.2%	10.1%	19.3%	73.0%
Hungary	18.0%	41.6%	23.2%	64.8%	17.2%
Iceland	16.1%	***4.8%***	4.7%	***9.5%***	74.4%
Ireland	60.3%	14.0%	4.6%	18.7%	21.0%
Italy	38.7%	20.6%	18.3%	38.9%	22.4%
Latvia	61.9%	20.7%	10.5%	31.3%	6.9%
Lithuania	56.6%	21.3%	16.4%	37.7%	5.7%
Luxembourg	***73.8%***	21.1%	***0.2%***	21.4%	4.8%
Netherlands	34.1%	48.9%	11.5%	60.4%	5.5%
Norway	11.3%	6.8%	3.6%	10.4%	***78.3%***
Poland	35.1%	33.0%	***28.7%***	61.7%	***3.2%***

Country	% Cattle	% Pigs	% Poultry	% Pigs + poultry	% Other animal species
Portugal	22.5%	35.4%	21.7%	57.0%	20.5%
Romania	29.8%	17.7%	14.5%	32.3%	37.9%
Slovakia	38.7%	22.9%	23.0%	45.9%	15.5%
Slovenia	55.1%	10.6%	22.3%	32.9%	12.0%
Spain	12.2%	49.7%	11.1%	60.8%	27.0%
Sweden	37.1%	24.6%	13.0%	37.6%	25.4%
Switzerland	59.1%	25.2%	8.6%	33.8%	7.1%
United Kingdom	25.1%	11.0%	16.1%	27.2%	47.8%

Blue and italics highlight the lower percentage value per column. Red and italics the highest percentage per column.

The mean % of cattle production in 2016 is 36.3% with a minimum of 7.6% (Greece) and a maximum of 73.8% (Luxembourg).

The mean of the percentage of pig production in 2016 is 28.7% with a minimum of 4.8% (Iceland) and a maximum of 73.3% (Denmark).

The mean % of poultry production in 2016 is 13.0% with a minimum of 0.2% (Luxembourg) and a maximum of 28.7% (Poland).

The mean % of the combined sales of pigs and poultry is 41.7% with a minimum of 9.5% (Iceland) and a maximum of 78.3% (Denmark).

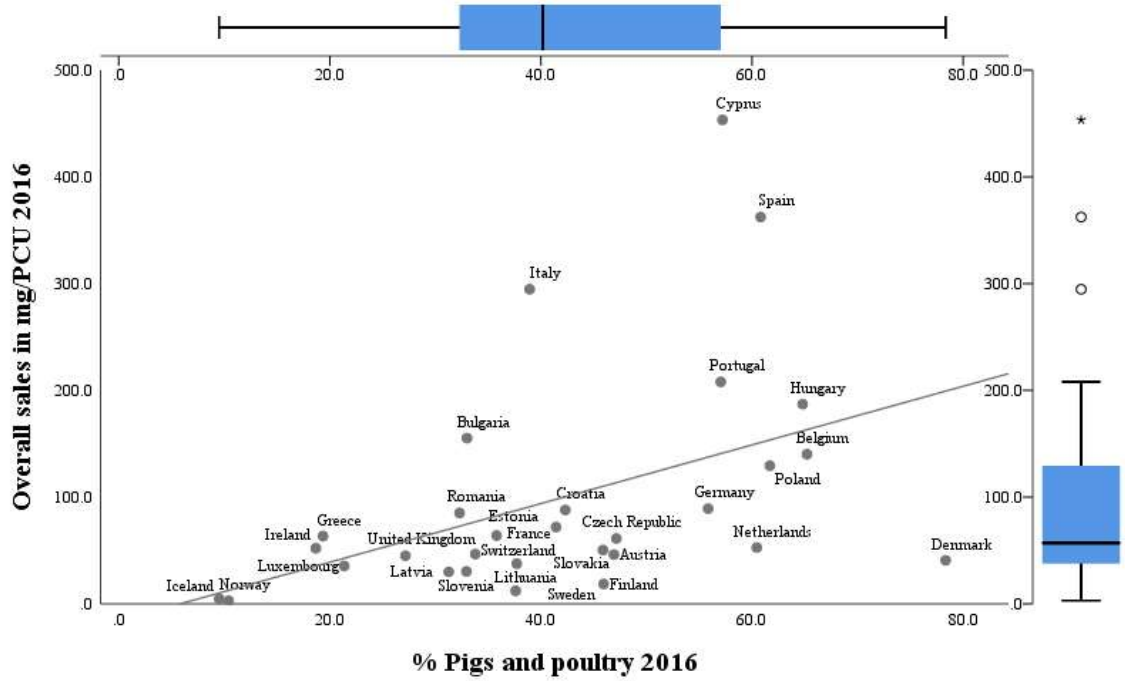


Figure 83. Sales of antimicrobials in mg/kg PCU in 2016 *vs* production of pigs and poultry in 2016.

The linear correlation of the overall sales in mg/kg PCU in 2016 *vs* the percentage of pigs and poultry produced in 2016 is significant, but not at the cut-off point of the Bonferroni correction (p = 0.015, 2-tailed). The Pearson correlation is moderate (0.439).

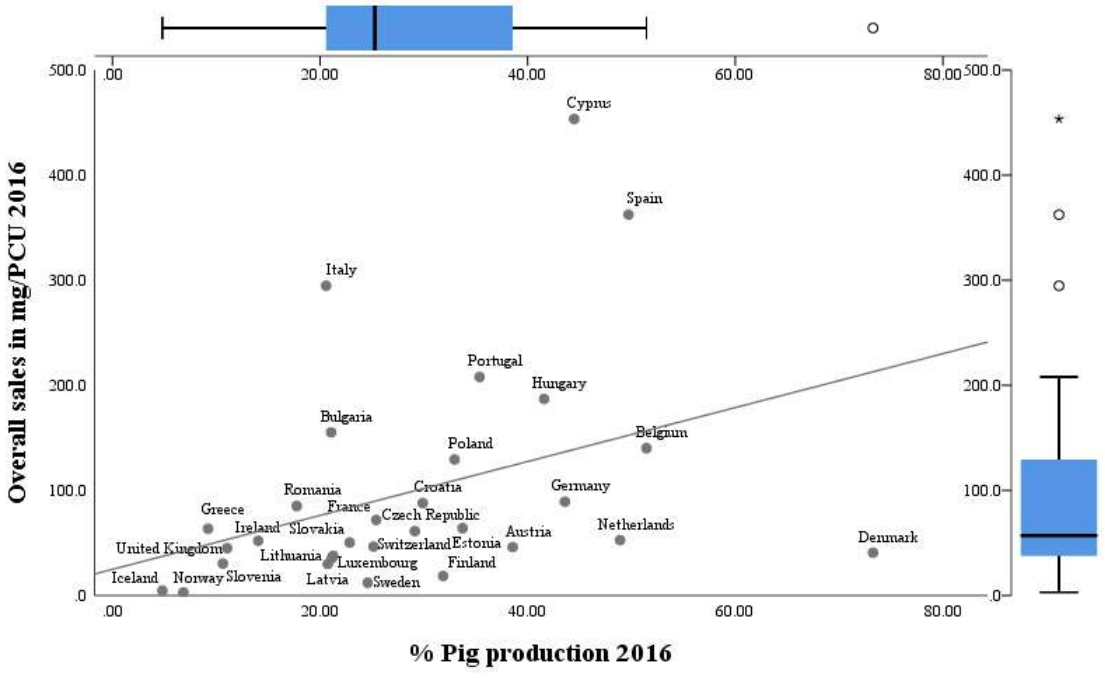


Figure 84. Sales of antimicrobials in mg/kg PCU in 2016 *vs* production of pigs in 2016.

The linear correlation of the overall sales in mg/kg PCU in 2016 *vs* the percentage of pigs produced in 2016 is significant but not at the cut-off point of the Bonferroni correction (p = 0.043, 2-tailed). The Pearson's correlation is weak (0.372).

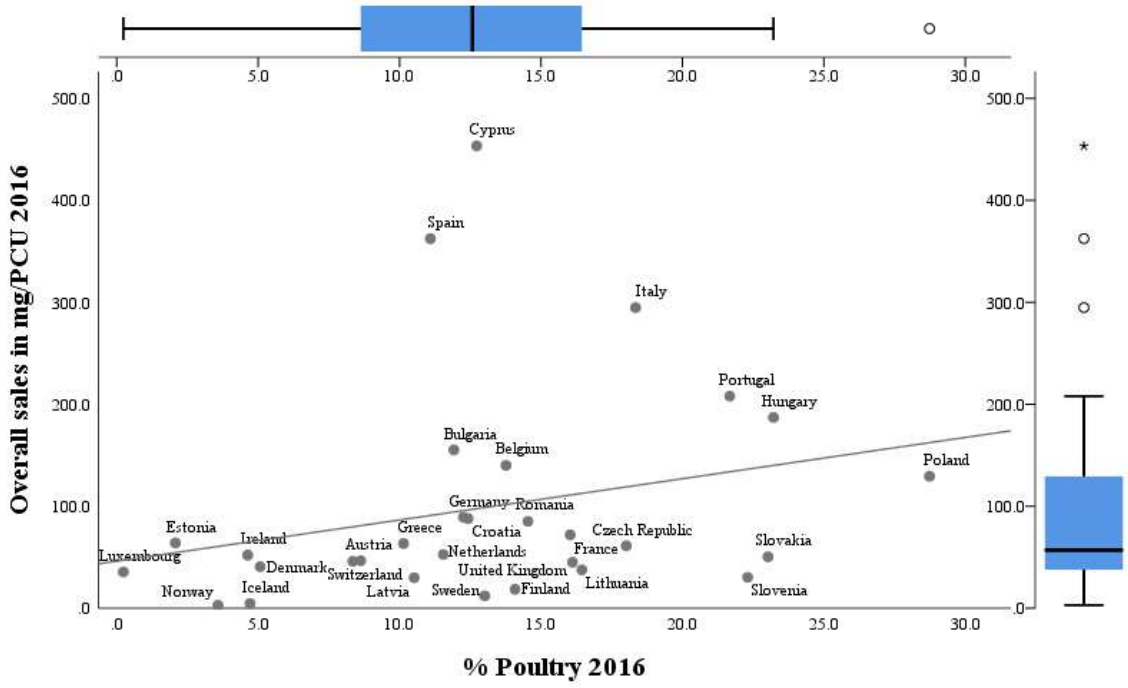


Figure 85. Sales of antimicrobials in mg/kg PCU in 2016 *vs* production of poultry in 2016.

The Pearson's linear correlation of the overall sales in mg/kg PCU in 2016 *vs* the percentage of poultry produced in 2016 is not significant (p = 0.257, 2-tailed).

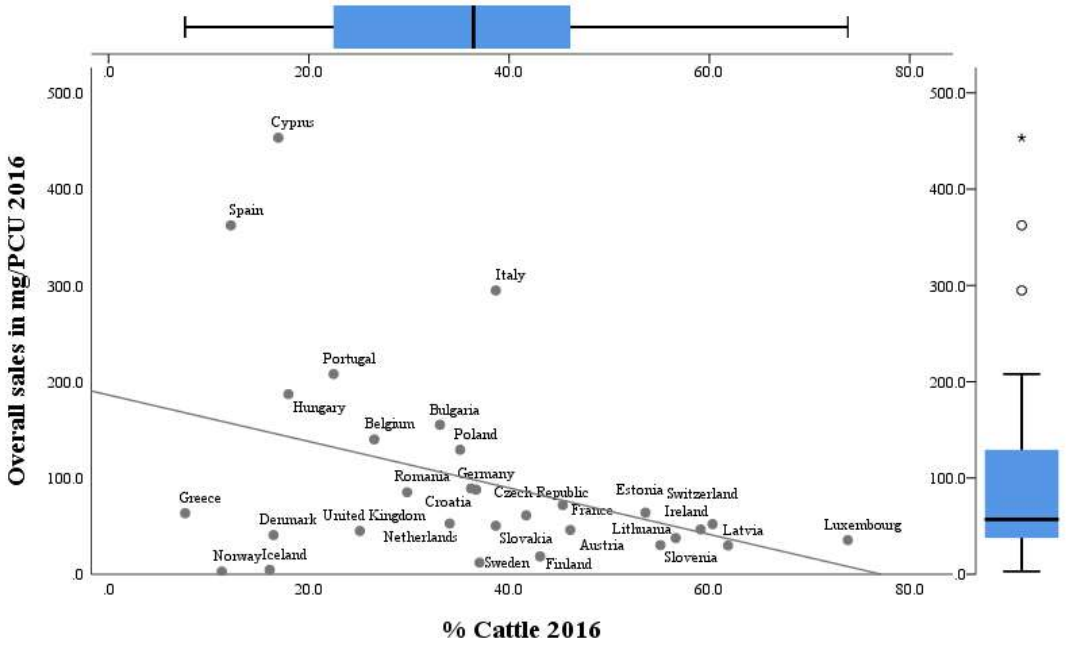


Figure 86. Sales of antimicrobials in mg/kg PCU in 2016 *vs* production of cattle in 2016.

The linear correlation of the overall sales in mg/kg PCU in 2016 *vs* the percentage of cattle produced in 2016 is significant at the 0.05 level but not at the cut-off point of the Bonferroni correction (p = 0.035, 2- tailed). The Pearson correlation is weak (nearly moderate) (-0.386).

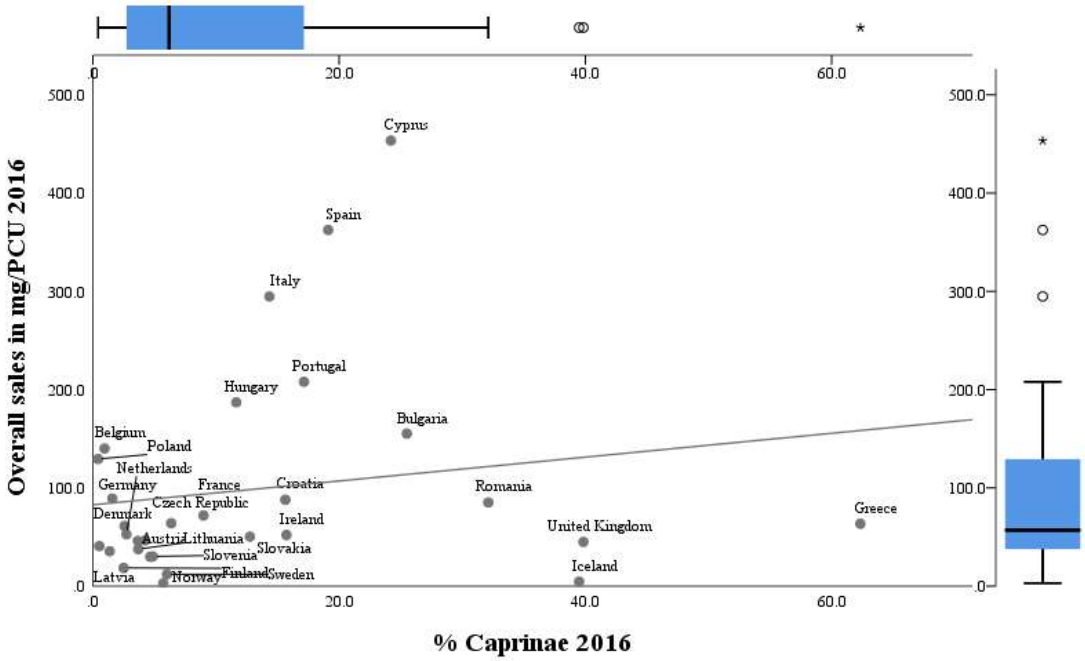


Figure 87. Sales of antimicrobials in mg/kg PCU in 2016 *vs* production of caprinae (sheep and goats) in 2016.

The Pearson's linear correlation of the overall sales in mg/kg PCU in 2016 *vs* the percentage of caprinae (sheep and goats) produced in 2016 is not-significant (p = 0.382, 2-tailed).

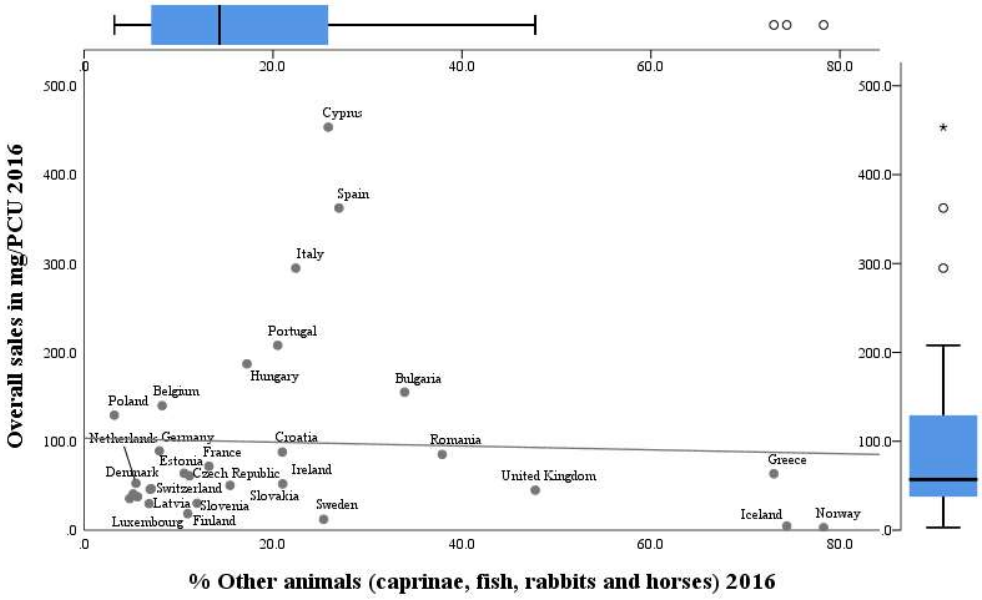


Figure 88. Sales of antimicrobials in mg/kg PCU in 2016 *vs* production of other animals (caprinae, fish, rabbits and horses) in 2016.

The Pearson's linear correlation of the overall sales in mg/kg PCU in 2016 *vs* the percentage of other animals (caprinae, fish, rabbits and horses) produced in 2016 is not-significant (p = 0.824, 2-tailed).

9.6.1. The animal species produced is associated with the variation of sales of antimicrobials during the years 2016-2010 (2011 or 2012)

The variation in sales of antimicrobials during the period 2016 to 2010 (2011 or 2012) was analysed with the intention to identify if a reduction or increase in antimicrobial use could be linked to the proportion of pigs and poultry produced in a country.

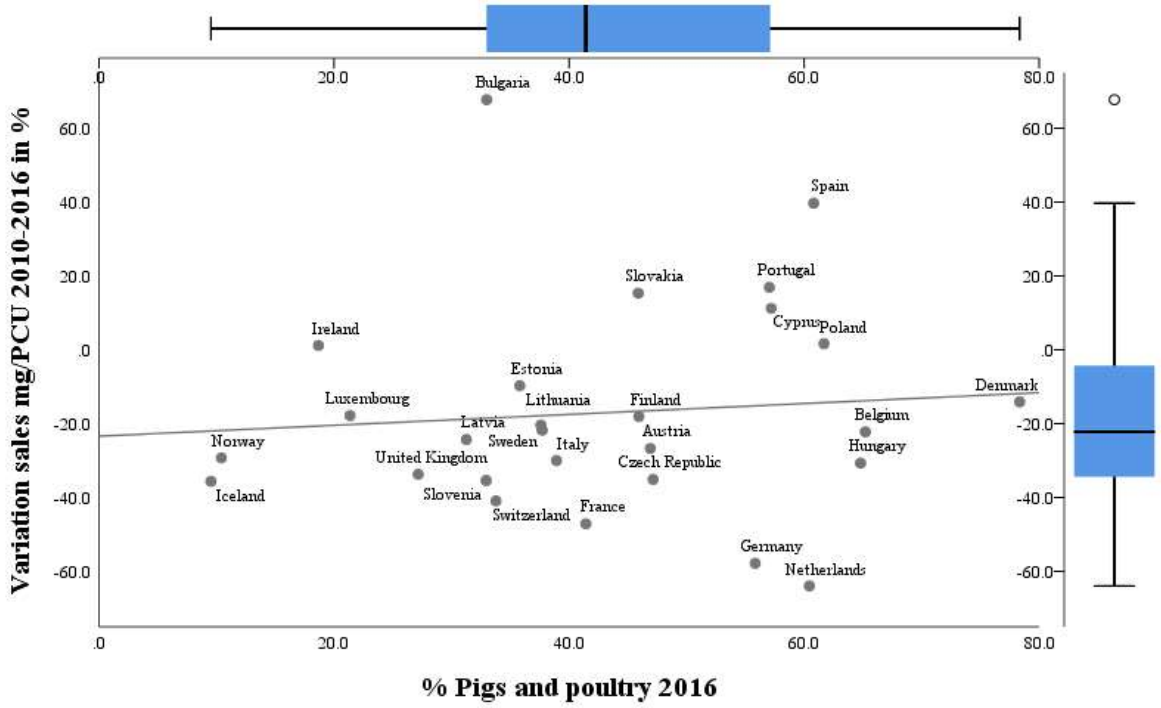


Figure 89. Variation on sales of antimicrobials in mg/kg PCU in 2016 *vs* production of pigs and poultry in 2016.

The Pearson's linear correlation of the variation of sales in mg/kg PCU of the years 2016 to 2010 (2011 and 2012) *vs* the percentage of pigs and poultry produced in 2016 is not-significant (p = 0.295, 2-tailed).

9.6.2. The percentage of oral forms for group treatment is associated with the animal species produced in 2016

The possible correlation between the pig plus poultry production (the most intensively produced animal species) and the use of oral forms for group treatment was analysed.

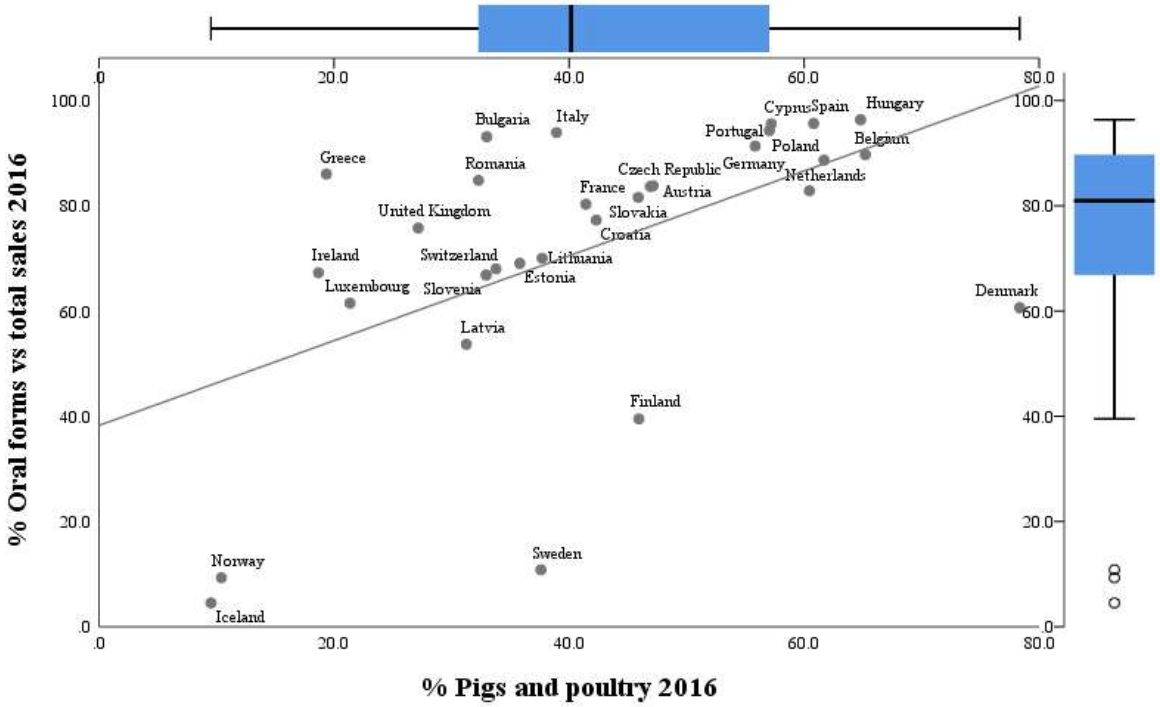


Figure 90. Percentage of oral forms *vs* production of pigs and poultry in 2016.

The Pearson's linear correlation of the percentage of oral forms *vs* production of pigs and poultry in 2016 is significant at the 0.01 level but not at the cut-off point of the Bonferroni correction ($p = 0.002$, 2-tailed). The Pearson correlation is moderate (0.538).

9.7. The average temperature in a country is correlated with the overall use of antimicrobials in animals in the country

The influence of temperature and rain on the overall AMC has been studied to find its possible correlation with the sales of antimicrobials. The results in mg/kg PCU of antimicrobials sold during the year 2016 were used as the reference.

Table 26. Mean temperature by country (2016) for which data are available on sales of antimicrobials for animals use.

Country	Mean temperature (C)*	Country	Mean temperature (C)*
Austria	7.0	Italy	13.5
Belgium	9.0	Latvia	6.0
Bulgaria	9.6	Lithuania	6.2
Croatia	12.5	Luxembourg	8.0
Cyprus	18.7	Netherlands	9.3
Czech Republic	6.8	Norway	4.3
Denmark	7.5	Poland	6.9
Estonia	5.5	Portugal	15.7
Finland	2.7	Romania	8.4
France	11.2	Slovakia	6.2
Germany	7.8	Slovenia	7.7
Greece	16.9	Spain	15.5
Hungary	10.0	Sweden	4.7
Iceland	3.4	Switzerland	6.0
Ireland	9.6	United Kingdom	9.3

Data obtained from http://www.weatherbase.com

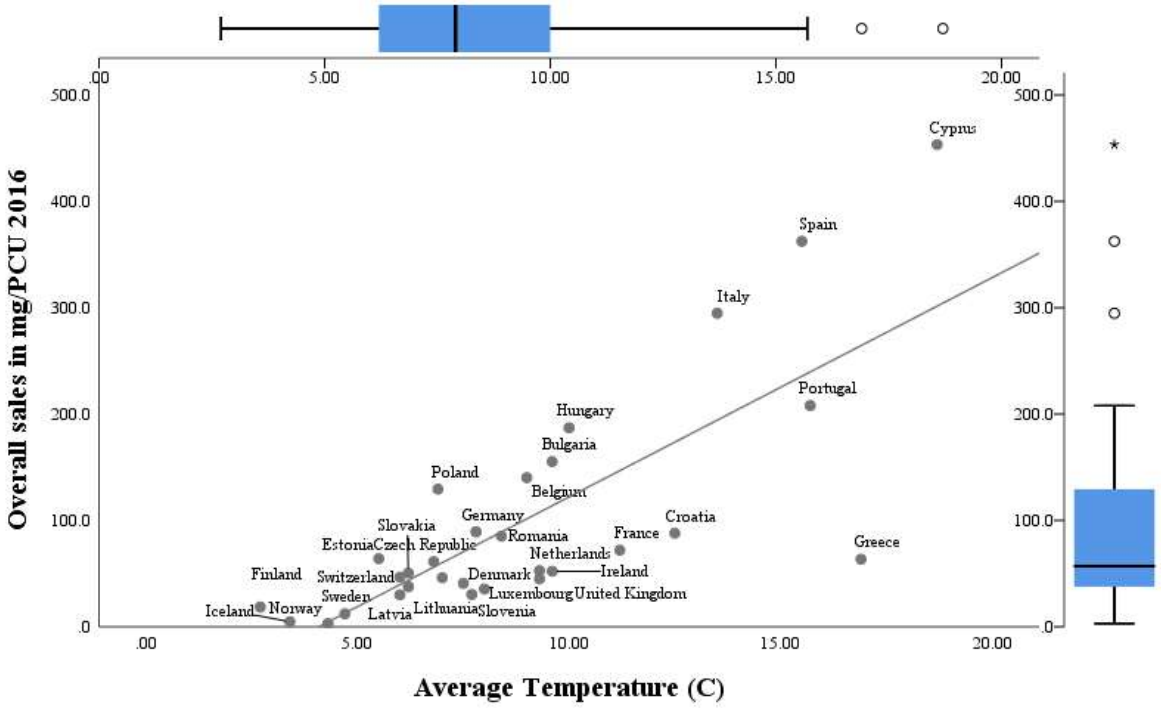


Figure 91. Sales of antimicrobials in mg/kg PCU in 2016 *vs* average temperature in the country.

The linear correlation of the overall sales in mg/kg PCU in 2016 *vs* the average temperature in the country is significant at the cut-off point of the Bonferroni correction (<0.00147 level, 2-tailed). The Pearson correlation is strong (0.768).

Similar estimations were made with the Average High Temperature (C) and Average Low Temperature (C) that were compared with the overall sales in mg/kg PCU in 2016. The result was in both cases that the correlation is significant at the cut-off point of the Bonferroni correction (<0.00147 level, 2-tailed). For the Average High Temperature, the Pearson correlation is strong (or nearly very strong) (0.788), and for the Average Low Temperature, the Pearson correlation is strong (0.727). Those correlations confirm the strong correlation between AMC and temperature.

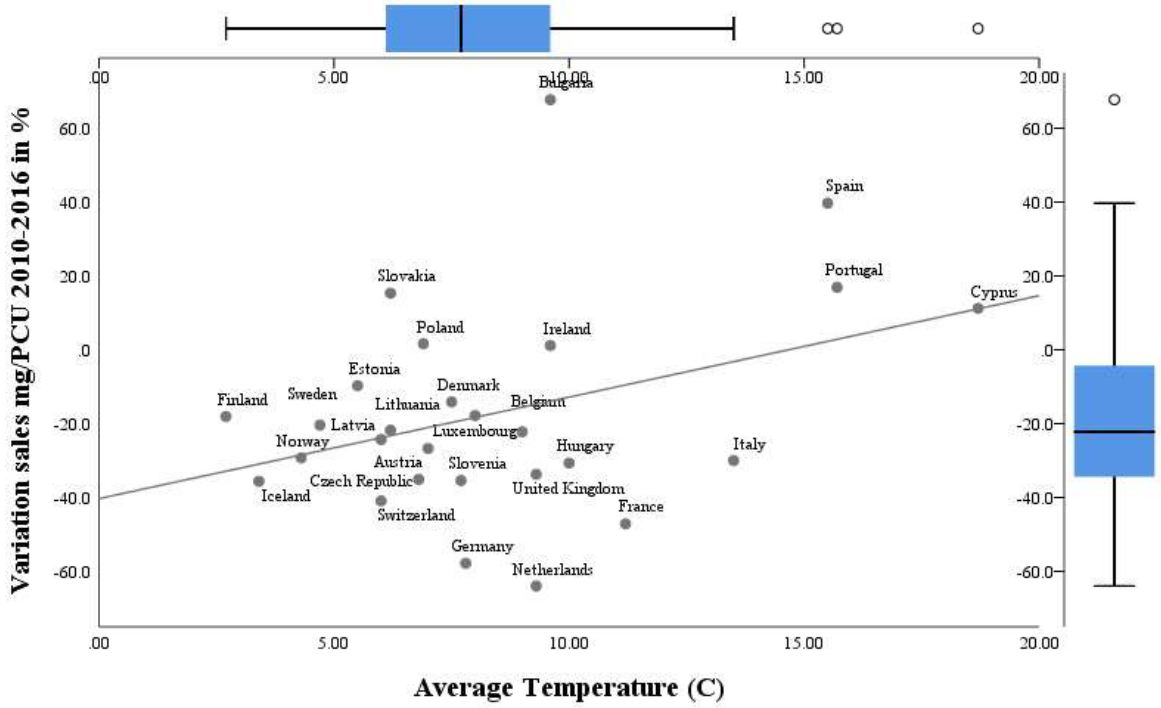


Figure 92. Variation on sales of antimicrobials in mg/kg PCU in 2016 *vs* average temperature in the country.

The linear correlation of the variation of sales in mg/kg PCU of the years 2016 to 2010 (2011 and 2012) *vs* the average temperature is not significant (p = 0.061, 2-tailed).

Other correlations were also analysed, including average precipitation and, e.g. HPCIAs and temperature.

An estimation was made with the Average Precipitation (mm) versus the sales in mg/kg PCU in 2016. The correlation is not significant at the 0.05 level.

The linear correlation of the sales of polymyxins in 2016 *vs* the average temperature is significant at the cut-off point of the Bonferroni correction (<0.00147 level, 2-tailed). The Pearson correlation is strong (0.664).

9.8. Pearson's linear correlations

As partially reported above, to detect possible statistical associations between pairs of continuous indicators, Pearson's linear correlations were produced for all of them.

Table 27. Variables considered in the correlation analysis subdivided into those corresponding to values of the year 2010, of the year 2016 and of the variation between both dates.

Values of the year 2010	Values of the year 2016	Variation between both dates
Overall sales in mg/kg PCU 2010	Overall sales in mg/kg PCU 2016	Variation sales mg/PCU 2010-2016 in %
3-4 gen. cephalosporins (mg/kg PCU) 2010	3-4 gen. cephalosporins (mg/kg PCU) 2016	% Δ Cephalosporins 2016 to 2010
All quinolones (mg/kg PCU) 2010	All quinolones (mg/kg PCU) 2016	% Δ All quinolones 2016 to 2010
Polymyxins (mg/kg PCU) 2010	Polymyxins (mg/kg PCU) 2016	% Δ Polymyxins 2016 to 2010

Table 28. Variables considered in the correlation analysis subdivided into value in 2016 and the percentage of the value in 2016.

Total (in 2016)	Percentage of the value in 2016
Total sales (tonnes) 2016	% Oral forms *vs* total sales 2016
Premix (mg/kg PCU) 2016	% Premixes *vs* total sales 2016
Oral powder (mg/kg PCU) 2016	% Oral powders *vs* total sales 2016
Oral solution (mg/kg PCU) 2016	% Oral solution *vs* total sales 2016
Injection (mg/kg PCU) 2016	% Injectables *vs* total sales 2016
	% Individual treatments *vs* total sales 2016
PCU (1,000 Tonnes) 2016	% Pigs 2016
Average Temperature (C)	% Poultry 2016
Average High Temperature (C)	% Cattle 2016
Average Low Temperature (C)	% Caprinae 2016

Total (in 2016)	Percentage of the value in 2016
Average Precipitation (mm)	% Other animals (caprinae, fish, rabbits and horses) 2016
	% Pigs and poultry 2016

The final matrix correlation is subdivided into six tables (Table 30 to Table 35), which are included in the Annexes

Annex I - Pearson linear correlations. The following scheme shows the layout of the six tables:

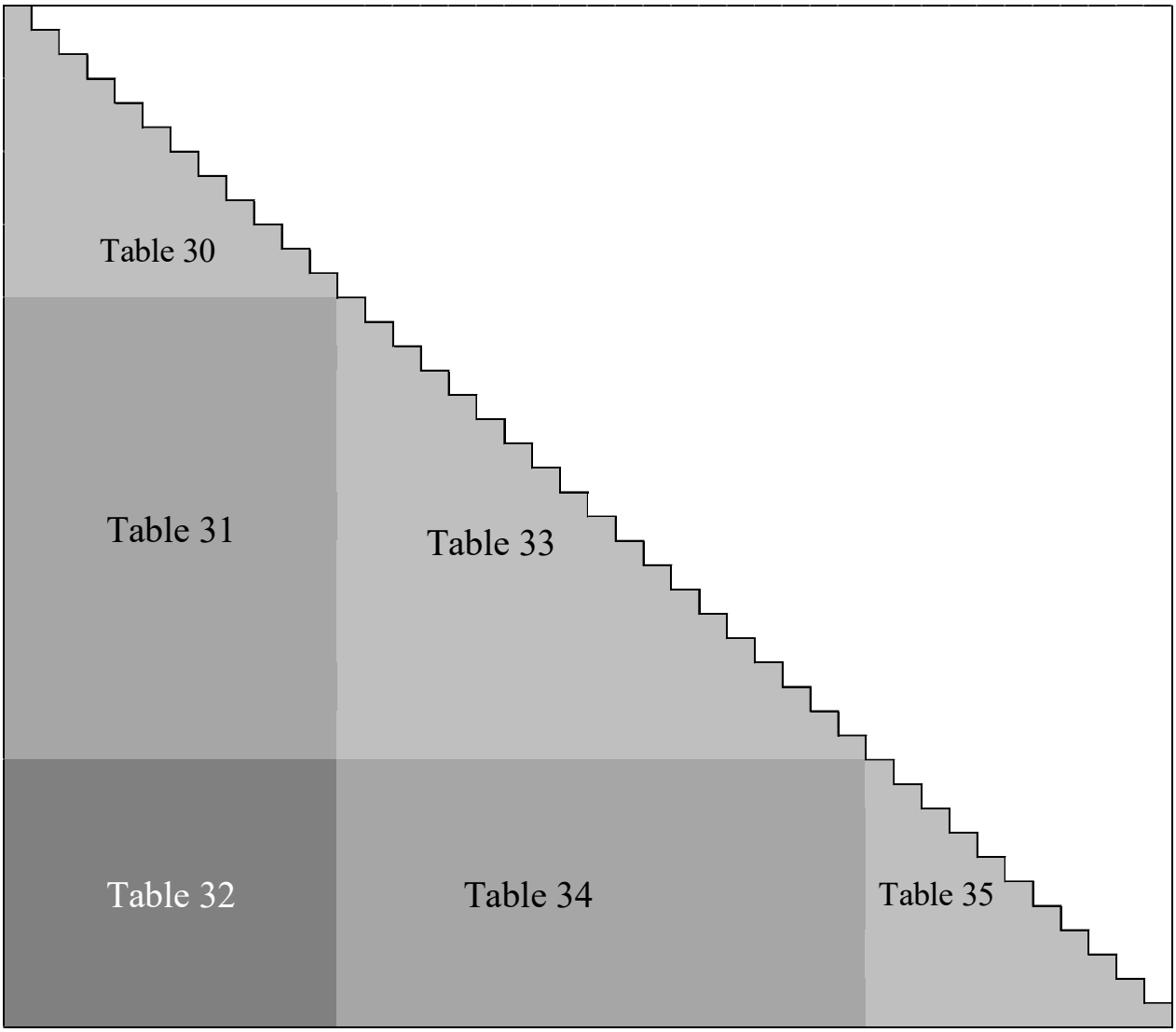


Figure 93. Schema of the six tables with the correlations of the data.

The p values were corrected using Bonferroni correction for multiple tests, given the large number of relationships analysed. Bonferroni's correction decreases the level of significance and the probability of incorrectly rejecting a null hypothesis. The procedure reduces the number of correlations for which the result is considered significant.

$$Bonferroni\ correction = \frac{Standard\ significance\ value}{n \text{ pairs of } variables}$$

$$Bonferroni\ correction = \frac{0.05}{24} = 0.00147$$

The Bonferroni's significant correlations are identified by blue coloured cells in the Annex I (Table 30 to Table 35) and listed in Table 36 of the same Annex.

9.9. Summary of the results

Table 29. Summary of the results obtained.

	Summary of the hypothesis	Result	Comments
1	Between 2010 and 2016, the use of antimicrobials in animals in the EU/EEA has been reduced in a statistically significant manner.	The decrease in sales of antimicrobials for food-producing species for 27 countries, between 2010 and 2016 is statistically significant (< 0.005, one-tailed).	Mean decrease for the period 17.1%
2.A.I	Reduction on % of the use of 3rd and 4th generation cephalosporins	The % variation of 3rd and 4th generation cephalosporins in mg/kg PCU between 2010 and 2016, by one-sample t-test, is not statistically different from zero (p = 0.381).	Wilcoxon signed-ranks test
2.A.II	Reduction on % of the use of quinolones	The % variation of quinolones in mg/kg PCU 2010 (or 2011, 2012) to 2016 is not statistically different from zero (p = 0.055).	
2.A.III	Reduction on % of the use of polymyxins	The % variation of polymyxins expressed as mg/kg PCU between 2010 and 2016, is not statistically different from zero (p = 0.442).	
3	The high percentage of oral forms of antimicrobials is associated with overall sales of antimicrobials	There is a strong Pearson's linear correlation between the % of oral forms *vs* total sales of antimicrobials (year 2016) with the overall sales in mg/kg PCU in 2010 (0.655), and a moderate correlation of the same % with the overall sales in 2016 (0.585).	
4	Collecting data on use by animal species associated with low sales	The comparison of the sales of antimicrobials (mg/kg PCU) in 2016 depending on collecting or not data by	Mann-Whitney U test

	Summary of the hypothesis	Result	Comments
		animal species, using the Mann-Whitney U test, does not allow concluding differences between them (p = 0.539, 2-tailed). The comparison of the variation of AMC (mg/kg PCU) between the years 2010 to 2016 depending on collecting or not data by animal species, using the Mann-Whitney U test, does not allow concluding differences between them (p = 0.059, 2-tailed).	Mann-Whitney U test
5	Collecting data on antimicrobial sales before 2007 associated with low sales	Non-parametric two independent test (Mann-Whitney U test) was applied, concluding that the differences between both groups are significant (p = 0.002, 2- tailed). The Mann-Whitney U test allows us to conclude that the increase of sales in both groups is statistically different (p = 0.006, 2-tailed).	Mann-Whitney U test Mann-Whitney U test
6	The animal species produced is associated with the overall sales of antimicrobials	The linear correlation of the overall sales in mg/kg PCU in 2016 *vs* the percentage of pigs and poultry produced in 2016 is significant (p = 0.015, 2-tailed). The Pearson correlation is moderate (0.439) not at the cut-off point of the Bonferroni correction. The Pearson's linear correlation of the percentage of oral forms *vs* production of pigs and poultry in 2016 is significant at the 0.01 level (p = 0.002, 2-tailed). The Pearson correlation is moderate (0.538).	
7	The average temperature in a country is correlated with the overall use of antimicrobials	The linear correlation of the overall sales in mg/kg PCU in 2016 *vs* the average temperature in the country is significant at the 0.01 level (p<0.0005, 2-tailed). The Pearson correlation is strong (0.768).	

Discussion

10. Discussion

10.1. Introduction

Use of antimicrobials in animals can lead to resistant bacteria which can result in failure in the treatment of important diseases in humans and animals [2, 23, 155, 158-160], those antimicrobials will also be released to the environment which might increase resistance of the bacteria surrounding us [90, 216, 218].

In a WHO commissioned a rapid systematic review of evidence to examine whether limiting the use of antimicrobials in food animals decreases AMR in animals and in humans provides evidence that limiting antimicrobials given to animals reduces AMR in animals. The review suggests that withdrawing antimicrobials in food animals results in decreased AMR in humans [222].

In the EU sales of antimicrobials for use in animals exceeds that of antimicrobials for use in humans [6, 7].

The EC overview report on measures to tackle AMR through the prudent use of antimicrobials in animals [32] indicates that substantial reductions in antimicrobial use have been achieved in the EU/EEA following the adoption of prudent use policies that do not seem to adversely affect animal welfare, productivity or profitability. And that there is some evidence to suggest that using antimicrobials prudently may lead to reductions in levels of AMR.

Sales of antimicrobials for animal use have been collected for most EU/EEA countries for many years by the EMA project called the European Surveillance of Veterinary Antimicrobial Consumption (ESVAC). The ESVAC project had an early publication with data from a few countries dating back to the years 2005 to 2009 [195]. However, those data were aggregated data (i.e. not at package level).

It is not until 2010 when the project started to collect detailed data from each participating country. The project rapidly gained popularity, although the collection of sales data from animals was not compulsory in many State Members.

The ESVAC reports are available on the EMA website as well as an interactive database from which most of the data used in this report can be downloaded.

Participating in the project has been, and still is, on a voluntary basis in many EU MS as such requirement is not included in the current EU veterinary medicines regulation [148].

The new veterinary Regulation (EU) 2019/6 [1] will not only provide legal basis for the collection of sales data but also request MS to collect data at farm level in a stepwise approach that should result in data on consumption of antimicrobials collected not only for all food-producing animal species but also for companion animals, the new veterinary Regulation will not be implemented until January 2022. Further mandates (secondary legislation) to clarify the collection of antimicrobial data (and other areas related to AMR and use of antimicrobials in animals) have been drafted and should be published during the following months by the EC.

Thirty EU/EEA countries provide data to ESVAC in a harmonised manner providing an excellent opportunity to analyse the results of such data. Although all countries act under a similar regulatory environment (except Switzerland), each one of them have different national risk mitigation measures implemented. Most of the countries have national action plans on AMR [23] that include, in most cases measures to reduce AMC at overall level or by, e.g. setting detailed targets on AMC reduction [125, 127, 128, 130, 145, 223]. Implementation of these plans varies widely between countries as identified by the EC inspections on AMR, which report how some countries have

achieved significant reductions of antimicrobial use by e.g. having comprehensive AMR plans, involving the food-producing sectors, setting targets of AMC reduction or monitoring AMC at farm level [125, 128-131, 134], whilst others have not been so successful on reducing AMC.

This report analyses the variation in sales of antimicrobials for animal use between the years 2010 to 2016, overall and by some classes of HPCIAs and provides an additional analysis of the one provided by the ESVAC reports by considering correlations of the data available.

The hypothesis analysed range from studying the significance of the decrease in the use of antimicrobials in animals between the years 2010 to 2016 (overall and by some classes of antimicrobials), to study in detail the use of different classes of antimicrobials for group treatment. In addition to analysing the correlations of the early collection of data on antimicrobial use with the overall sales, the collection of data by animal species and the average temperature in a country. Finally, an overall analysis of all the possible correlations studied in the report is made.

10.2. Use of antimicrobials in animals in the EU/EEA between 2010 and 2016

Twenty-seven countries provided data to ESVAC for the years 2010 (2011 or 2012) to 2016, for which the increase or decrease of sales of antimicrobials for animal use could be measured.

Twenty-one countries had data available from 2010 (Austria, Belgium, Czech Republic, Denmark, Estonia, Finland, France, Hungary, Iceland, Ireland, Italy, Latvia, Lithuania, Netherlands, Norway, Portugal, Slovenia, Spain, Sweden, Switzerland and United Kingdom), 5 from 2011 (Bulgaria, Cyprus, Germany, Poland and Slovakia) and one from 2012 (Luxembourg).

Overall sales in mg/kg PCU for the years 2010 to 2016 as provided in Table 4, Table 5 and Figure 10 to Figure 17, indicate that the decrease in sales of antimicrobials for food-producing species for 27 countries, between 2010 and 2016 was statistically significant, from 117.6 mg/kg PCU (years 2010, 2011 or 2012) to 98.6 mg/kg PCU (2016) with a mean decrease of 17.1% on the sales during those years (standard deviation of 28.7% and a median of 22.2%).

In Table 4 it can be observed that 20 countries had a decrease in the % of sales in mg/kg PCU between the years 2010 (2011 or 2012) and 2016, with a maximum decrease of 64.0%, and 7 countries had an increase in the % of sales in mg/kg PCU during the same years. There was a maximum increase of 67.7% during the period from one country, although this increase can be partially attributed to a lack of reporting of sales during the initial years of the data collection [65]. Therefore, it can be concluded that the majority of EU/EEA countries show a significant decrease in antimicrobial use (see Figure 16 and Figure 17) during the analysed period.

This significant reduction was expected as during the last years many countries had initiated activities on antimicrobial reduction in the veterinary field [56, 59, 130]. The reduction is estimated in mg per kg of PCU, which takes into account the animal population susceptible to be exposed to antimicrobial use, i.e. the reduction is, generally, independent of the increase or decrease of animals produced in a year in a given country.

Most of the reductions can be attributed to the decisive action of some MS like the Netherlands [130], Germany [128] or France [127] which have made those responsible of the use of antimicrobials (veterinarians, farmers, operators...) to change attitudes towards the use of antimicrobials in animals. All those countries have set targets for the reduction of antimicrobial consumption.

The 2018 Eurobarometer on AMR [224] indicates that most respondents (85%) are aware that unnecessary use of antibiotics renders them ineffective, and a similar proportion (84%) know that you should only stop taking antibiotics after taking all of the prescribed doses as directed.

Some countries (e.g. Finland and Norway) maintain a high health status of their national herds through checks to prevent the introduction of certain conditions in animals entering the countries, i.e. limiting the entrance of animals that might require antibiotic use [32]. Improved biosecurity is also one of the main drivers that reduce the use of antimicrobials [32, 139].

The fact-finding missions on AMR in MSs [32] have certified that the combination of preventive measures allows to almost phase-out the use of antimicrobials in specific sectors (e.g. poultry and aquaculture). The ESVAC data seem to confirm those findings. This reduction of antimicrobial use in animals might seem a utopia, and not applicable through the whole EU/EEA, or even to all animal production systems, but it debunks the myth that in animal production AMs have to be used in a systematic, compulsory manner, and makes apparent that it should be possible to improve the production systems to dramatically reduce the AMC in animals, making valid the mantra: "Prevention Is Better Than Cure". When analysing the correlations between overall antimicrobial use in 2016 and the combination of poultry and pigs, or pigs and poultry on its own, we find that the correlations are moderate at maximum (0.439 for pigs plus poultry, 0.372 for pigs and not significant for poultry) but not at the cut-off point of the Bonferroni correction (see Table 32). Of all studied countries Denmark has the highest % of pig production (73.3%, see Table 25) whilst is one of the countries that belongs to the group of low countries consumption in mg/kg PCU (see Figure 13). The data showing an AMC reduction in Scandinavian countries confirm the effectiveness of policies of those countries to reduce AMC, which is based on involving all stakeholders, surveillance and decisive action like the banning of AGPs back in the mid-1990s.

Although direct comparison of sales of antimicrobials for use in food-producing species between countries should be done with caution and taking into account the context of those sales, including factors like the production systems, animal population and the antimicrobials authorised for animal use [86]. The differences between the sales of antimicrobials for use in animals in the country selling the most mg of antimicrobials per kg of animal produced in a given year (Cyprus, 453,4 mg/kg PCU, year 2016) *vs* the country with the least sales of antimicrobials per kg of animal produced (Norway, 2,9 mg/kg PCU, year 2016) - a difference superior to 150 times the use of antimicrobials between both countries - is striking.

The above-mentioned differences seem to justify that measures are taken by the countries with the highest consumption of antimicrobials per kilogram of animal produced in order to reduce the overall use of antimicrobials in animals. Many reports have detailed which measures work in order to reduce AMC [32, 46, 139], as well as many national plans on AMR from MS that detail which measures have been taken to reduce AMR [32, 99], some years ago these reports were confined mostly to the Scandinavian countries (notably from Denmark [55, 225], Norway [131] and Sweden [134], whereas nowadays there are plenty of reports from EU countries that have reduced their antimicrobial use from Western and Mediterranean Europe [31, 56, 58, 59, 82, 130].

As can be observed from Table 4, the countries that had sales above 200 mg/kg PCU in 2016 were Portugal, Italy, Spain and Cyprus. Of those countries, Italy had an important decrease in sales in the period 2010 to 2016 (-30%), whilst Spain (39.7%) Portugal (16.9%) and Cyprus (11.2%) had an increase during the same period. From the information available, although some of those countries have set action plans on AMR to improve prudent use of antimicrobials, overall national targets on reduction of antimicrobial use in animals (apart from those set on colistin by the EMA) were rarely introduced during the studied years.

According to the EC fact-finding missions [32], in some countries, the setting of targets was not done claiming limitations in the data available to appropriately set targets for antimicrobial use and to monitor progress as well as the lack of a legal basis for enforcing these targets. Some competent authorities expressed the view that targets should be set based on technical rather than political

criteria, in order to avoid problems of animal welfare amongst other reasons. The information available for the preparation of this report seems to suggest that setting targets on reduction of AMC has not impacted animal welfare.

In the case of Spain, there were some deficiencies on the initial data collection that could partially explain the increase in sales on the period 2010 to 2016, as detailed on the ESVAC report including data from the year 2014 [70]. Spain changed its system for collecting sales data in 2014, and it was identified that at least one company marketing antimicrobials (with some of the highest-selling veterinary medicinal products for 2014 and previous years), had not been reporting sales between 2011 and 2013. Other countries (e.g. Bulgaria and Slovakia) reported sales of antimicrobials for animals in the year 2011, which might be an under-reporting of sales during those initial years.

From the above described under-reporting during the initial years of data collection it can be assumed that the supposed increased of sales on the period 2011-2016 for Bulgaria (67.7%), Spain (39.7%) and Slovakia (15.3%) are overestimated, as a consequence it can be speculated that the reduction of antimicrobial use in animals in the period 2010 to 2016 could be higher than estimated by the ESVAC data.

In addition to the technical explanations provided above, some of the reasons for increases of antimicrobials sales can be found on the EC Directorate-General for Health and Food Safety reports [32] which provide detailed reports on visits to EU/EEA MS in relation to AMR, in some cases detailing very specific measures to reduce AMC (e.g. setting targets and collecting data on AMC at farm level).

In the case of the Bulgaria, the country that has increased the most the sales of antimicrobials between 2010 and 2016 (sales of 155.3 mg/kg PCU in 2016), the ECDC/EC one health report to discuss policies relating to AMR – using surprisingly direct language for an official EC report - indicates that there are numerous gaps and weaknesses in the approach towards tackling AMR in the country. Those deficiencies are both, in the veterinary and human health domains, which compare poorly with the situation in other MS [144]. The report stresses that there is, in particular, a significant lack of communication and collaboration between the veterinary, human health and environmental authorities in a One Health perspective and that there is no Inter-sectoral One Health Coordinating Mechanism on AMR. Furthermore, draft national action plans for animal health and for human health, which are in various stages of development, have been developed separately. It also indicates that in the veterinary sector awareness concerning AMR is generally very low and few effective initiatives have been taken by the national authorities to date, providing other priorities as the reason for the lack of activity as, e.g. dealing with cases of African Swine Fever.

The report from Bulgaria [144], in relation to the sales of antimicrobials for animals use the authorities account that a reported increase of 27% increase might represent incomplete reporting of sales and consumption in previous years, rather than an actual increase in AMC in 2016. Moreover, the data reported to ESVAC do not currently take account of antimicrobials brought into the country by veterinarians (under the cascade) or the use of human medicines in animals, which in fact could make the exposure of animals to antimicrobials worse than the data reported to the ESVAC project. The findings described in the report on the AMR situation in Bulgaria is of concern.

Spain has one of the most significant increases in sales of antimicrobials for animal use during the period 2010 to 2016, the report from the EC [123] indicates that Spain is currently taking measures to reduce AMC and that whilst it is too early to see how effective the Spanish AMR action plan will be, there are indications that significant reductions in the use of antimicrobials could be achieved without adversely affecting productivity and costs. These indications derive from a voluntary national initiative to reduce the use of colistin and from the individual efforts of farmers,

veterinarians and producer groups. Moreover, on the sales of antimicrobials for animal use the report confirms what has before been reported to the ESVAC project that according to the Spanish authorities the apparent upward trend in sales of antimicrobials during the last few years can be partly explained by previous underreporting. It also highlights that preliminary data indicates that sales of antimicrobials for use in animals have continued to rise by approximately 10% in 2015 and that there is little sign of a downward trend in 2016, apart from colistin, which is the subject of a national voluntary programme. In the case of Spain - although the EC report is encouraging - the high level of mg/kg PCU in 2016 (362.5 mg/kg PCU) is also worrisome, and one can only hope that the measures taken will result in a substantial reduction on overall AMC in the country. The Spanish National Plan against resistance 2019-2021 indicates that an agreement of the pig producers representing 80% of the producers has already achieved a reduction on the colistin consumption of 97,2% between the years 2015 to 2018, which is a remarkable achievement [226].

The above described under-reporting to the ESVAC project in a few countries is not uncommon during the first years of data collection and is what has led to add a disclaimer on the ESVAC reports [65, 71] indicating that it is generally agreed that it takes at least three to four years in order to establish a valid baseline for the data on sales of veterinary antimicrobial agents and that data from countries that have collected data for the first or even second time should be interpreted with due caution.

In a report from the Federations of Veterinarians of Europe (FVE) [208] it is indicated that species kept for food production, as well as the husbandry and management conditions of how these animals are reared, differs greatly between countries in Europe. This also applies to the relative proportion of the various animal species/sub-species/category of animals, the climate, epizootiology, the infectious disease and the availability of veterinary antimicrobial products and alternatives. As a result, indications to prescribe antimicrobials for and amounts used per species, vary greatly per species. All those factors have to be taken into account when comparing data on antimicrobial use in animals. The first ESVAC report already indicates that a major finding is the substantial difference in the prescribing patterns of veterinary antimicrobial agents between the countries and that these variations may be due to differences between the countries in the availability of veterinary antibacterial products on the market in those countries, prices, risk-management measures implemented, the veterinarians' prescribing behaviour, animal production systems (e.g. veal as opposed to beef cattle on pasture) and the general situation with regard to infectious diseases [61].

The decrease of sales of antimicrobials in the Netherlands, France and Germany as seen in Table 4 is truly remarkable, especially since all of them are countries with a big animal production, which results in a higher decrease of total antimicrobial use in tonnes per year. In 2016, all three countries had sales below 100 mg/kg PCU. The Netherlands and Germany have established antimicrobial data collection at farm level with benchmarking methods [128, 130, 198], in addition the Netherlands has reduced the use of 3rd and 4th generation of cephalosporins to levels that are close to zero, whilst France has an impressive AMR plan and set target on AMC reduction [227] and although France has not set systematic farm data collection at country level, it has been pioneering many aspects of the data collection of antimicrobials for many years, including collecting data on companion animals and working on different types of indicators of AMC like the Animal Level of Exposure to Antimicrobials, which is an indicator correlated to the percentage of animals treated relative to the total population and according to the French authorities is an

objective indicator of exposure to antimicrobial [40, 59, 228, 229]. All the mentioned three countries had set targets on reduction of antimicrobial consumption at national or farm level

The decrease of some countries in overall sales of antimicrobials like Italy (-30.0%) and Hungary (-30.7%) is also remarkable although they remain high consumers of antimicrobials (294.8 and 187.1 mg/kg PCU respectively), see Figure 26, but in any case it shows that MSs can indeed reduce AMC in animals.

It is also noteworthy that as shown in Table 4 all countries that already had a low consumption of antimicrobials for animal use have continued reducing their AMC during the period 2010 to 2016 (Iceland, -35.6%, Norway -29.3%, Sweden, -20.4%, Denmark, -14.1% and Finland, -18.1%). Most of those countries have had systems of data collection of antimicrobials for many years [61, 195]. Production systems that are mostly based on extensive production of animals (except Denmark) and highly structured animal production systems with high control of the animal health conditions. In Denmark, Sweden and Norway the decoupling of sales and prescription of antimicrobials (limitation of dispensing of veterinary medicinal products by veterinarians to non-profit sales) is considered a key part of their policy on reduced use of antimicrobials [125, 131, 134]. In Denmark approximately 75% of pigs are born in a Specific Pathogen Free environment [206], i.e. free of mycoplasmosis, pleuropneumonia, swine dysentery, mange, lice, porcine reproductive and respiratory syndrome, and atrophic rhinitis of pigs, which suggests that animals will require less antibiotics when growing by reducing the need for treatment of infectious diseases.

What seems to be a common theme in the countries that already have low sales of antimicrobials is the involvement of all stakeholders with the common objective to reduce AMC, i.e. to sort a societal problem – AMR – as a group, and not based exclusively on taking measures against individual actors. In some of those countries there is a very detailed data collection systems at farm level (e.g. Denmark), whilst in others data are collected exclusively at sales level (e.g. Iceland).

As an example, Germany has done a remarkable task of reducing their antimicrobial use from 211.5 mg/kg PCU in the year 2011 to 89.2 mg/kg PCU in the year 2016 (a reduction of 57.8%). Ungemach *et al.,* 2006 [138] highlight that acceptance of the guidelines for prudent antibiotic use by veterinarians as a vital tool to reduce the usage of antibiotics and the likely development of resistance. Guidelines on prudent use of veterinary antimicrobial drugs were established in 2000 and revised (at least) in 2010 [230], those guidelines include recommendations requiring a diagnosis based on an appropriate clinical examination and, if required, further diagnostic laboratory tests, taking into account the immune status of the animals, stock-specific aspects and other experiences and knowledge and to keep the duration of treatment kept to the minimum required for the therapy.

In 2017 a second interim report of the German Antimicrobial Resistance Strategy was published [231]. The report details the Veterinary Consumption of Antibiotics project providing details on how the consumption of antimicrobials is measured in the country which includes the average number of times a farm animal is treated with antibiotics, the types of active substances, their volumes and the frequency of their application. The benchmarking system is established to collect data at farm level and identify those farms where the antibiotic consumption is higher than in similar farms, i.e. a benchmarking system to minimise the use of antibiotics in farm animals. The system centres on comparative analysis of individual treatment frequencies in farms compared to average nationwide figures. The analysis is broken down into different farm animal species and age brackets. Where treatment frequencies of farms exceed the reference figures, livestock owners are required to consult a veterinarian to establish the cause. Analysis of the cause may result in mandatory measures to reduce the consumption of antibiotics in the enterprise. If the index of a farm is above a defined threshold, the farmer and/or the competent authority have to take measures

to reduce the need for antibiotics [58]. The reduction of individual treatment frequencies lowers the nationwide figures calculated on the basis of the individual figures. All those combined actions have resulted in an impressive antibiotic reduction in the country.

Figure 18 shows a big dispersion of results between those countries with high antibiotic consumption in 2016 with respect to the variation of their sales during the period 2010 to 2016, this shows that the countries participating in the ESVAC project had not applied a common policy of antibiotic reduction during the period studied, this has been confirmed by the fact-finding missions on AMR from the EC [32] and that the correlation between the sales of antimicrobials in mg/kg PCU in 2016 and the variation on sales of antimicrobials in 2010 to 2016 is only moderate (0.467) and significative at the 0.05 level but not at the cut-off point of the Bonferroni correction.

From the fact-finding reports from MS on their activities on AMR, including the reduction of AMC, it can be concluded that a few MSs have set up systems with targets to reduce AMC, including Belgium, Denmark, France, Italy and Netherlands [31, 127, 141, 142, 145, 223] or, e.g. benchmarking systems to monitor the use of antimicrobials at farm level, or set up national treatment guidelines like Denmark, the Netherlands and Germany [125, 198, 231]. This lack of harmonised measures has not led to a homogenous reduction of AMC in all countries.

However, measures to reduce AMC take time. Many countries that will have initiated some of the previously mentioned measures might not yet seen the results reflected in their sales, and this hopefully will be the case of, e.g. Spain [123].

The new veterinary Regulation (EU) 2019/6 [1] (see 5.10.5. , page 42 for further details) includes a series of measures, including strengthening of the prudent use of antimicrobials by e.g. avoiding their routine prophylactic and metaphylactic use, a use that has been identified in some countries as one of the major reasons for high use of antimicrobials or restrictions on the use of antimicrobials that are of critical importance for preventing or treating life-threatening infections in humans, which in cases like colistin could result in an important reduction of antimicrobial use. Overall, those legal requirements should result in a sharp decrease in AMC in most EU/EEA countries, at least in those with middle or high consumption of antimicrobials, and although the implementation of the mentioned regulation will not start until January 2022, EU/EEA MSs should already be preparing for it.

The analysis of antimicrobial sales data carried out in this work is in line with the ESVAC report with data from 2016 [71], which analyses the aggregated variation on sales of overall sales of antimicrobials for 25 countries between 2011 and 2016, and finds a decrease in the sales from 162.0 (year 2011) to 129.4 (year 2016) mg/kg PCU, a decrease of 20% of the sales of all antimicrobials in mg/kg PCU.

The antimicrobials consumed by animals are partly dispersed in the environment therefore, the presence of those substances in the environment is a rising concern, the EC on its evaluation of the action plan against the rising threats from AMR [20, 21, 175] notes that the presence of resistant micro-organisms in the environment and their impact on development and spread of AMR in the environment is still considered a knowledge gap.

Recently there have been some relevant publications about the presence of antibiotics in the environment - in e.g. sewage, or Antarctica - showing a worrisome presence of antibiotics in remote areas of the environments as well as systematic differences in abundance and diversity of AMR genes between the continents of the world [232, 233], although it is not yet clear which is the hazard for the environment or animal or public health of the presence of those AMR genes, is undoubtedly of concern.

Although antibiotics consumed by animals and later on released to the environment might be degraded with time, the accumulation of mg/kg PCU of antibiotics over the years 2010 (2011 or 2012) to 2016, shown in Figure 21, raises questions when considering the accumulation of antibiotics in the environment, either for its impact on public health or because of the accumulation of antimicrobials as a source of contamination into the environment. If trying to do an analysis of the spatial distribution of antimicrobials in environmental factors such as the size of a country or livestock density, which should be considered [217].

As an example of the possible exposure of the environment from antimicrobials used in animals, the sum of the sales of antimicrobials for animal use per mg/kg PCU between the years 2011 and 2016 in Cyprus (surface of 9,251 km^2) is about 2,500 mg/kg PCU (i.e. 407.6, 396.5, 425.8, 391.5, 434.2 and 453.4 mg/kg PCU) (see Table 4 for further details), or 279.6 tonnes (i.e. 51.8, 45.0, 47.9, 41.7, 46.9 and 46.3) (see Table **7**). The addition of the sales of antimicrobials for animal use per mg/kg PCU during the same period in Norway (surface of 385,170 km^2) is about 20 mg/kg PCU (i.e. 3.7, 3.8, 3.7, 3.1, 2.9 and 2.9 mg/kg PCU) (Table 4), or 36.9 tonnes (i.e. 6.2, 7.1, 6.6, 5.8, 5.6 and 5.6) (see Table 7). This results in an important difference between the amounts of antimicrobials to which the environment could potentially be exposed in those two countries.

The total amount of tonnes of antimicrobials sold for use in animals might be of relevance for the environment. Sales of antimicrobials by weight (tonnes) should be analysed with a proper indicator (e.g. animal production) and adequate context [73, 77, 187, 204]. In any case, considering that those sales in tonnes might impact public health, and also the increasing concerns about the presence of antimicrobials in the environment, is relevant to analyse the sales of antimicrobials in tonnes as a proxy for the release of those substances in the environment and its possible impact on animal and public health.

Figure 24, Figure 25 and Figure 26 show total sales (tonnes) by countries, divided into 3 groups (depending on the maximum sales in 2016) and in alphabetical order within each group: *i)* countries with lower consumption (up to 40 tonnes), *ii)* countries with middle consumption (40-300 tonnes) and *iii)* countries with higher consumption (more than 300 tonnes).

The total reduction of 3 of the biggest EU countries (Italy, Germany and France) during the period 2010 (or 2011) and 2016 amounts to a total of 2237.7 tonnes, whilst the increase during the period of just one country during the period 2010 to 2016 accounts for 920 tonnes.

Depending on the consumption in mg/kg PCU and tonnes of a country, it can be observed that:

- Countries with a small use of antimicrobials have continued to reduce their use, even if such use is already much smaller than countries with higher use.
- Countries like Austria, Belgium and the Czech Republic, in the middle group, have an active policy on antimicrobial reduction [71, 81, 143, 220] and the data show that this has paid back through the years on an overall reduction of the tonnes consumed.
- Whilst sales in tonnes of antimicrobials in some of the biggest countries show a remarkable decrease (e.g. France, Germany and Italy), this is not the case for all of them, most remarkably Spain (see Table **7**).

Others have analysed the sales of antimicrobials in tonnes and its relationship with resistance, not only in animals but also in humans. The second joint inter-agency antimicrobial consumption and resistance analysis report (JIACRA II) [7] includes a chapter on consumption of antimicrobials in humans and food-producing animals which indicates that in 2014, 3,821 and 8,927 tonnes of active substance of antimicrobials were sold (or used) for consumption in humans and food-producing animals, respectively, in 28 EU/EEA MS.

In addition to noting the high proportion of antimicrobials used in animals (57.2%) versus those used in humans (42.8%), is also to note that the above-mentioned reduction in 3 countries (Italy, Germany and France) in the period 2010 to 2016 (2237.7 tonnes) is more than half the consumption of antimicrobials in human medicines in 28 countries during the year 2014. From the point of view of the release of antimicrobials into the environment it shows how a reduction on antimicrobial use in animals could reduce exposure of the environment to antimicrobials and also reduce exposure of animals and humans, to those antimicrobials.

The Pearson's linear correlation of sales of all antimicrobials in mg/kg PCU in 2016 *vs* sales in tonnes of antimicrobials in 2016 is moderate (0.578) and significant at the cut-off point of the Bonferroni correction (<0.00147 level, 2-tailed), this seems to suggest that countries with high consumption of antimicrobials in total tonnes also tend to have high consumption of antimicrobials per kg of animal produced.

The New Veterinary Regulation (EU) 2019/6 [1] includes a requirement for MS and the EMA to ensure quality and comparability of the data on sales and use of antimicrobials in animals. It also requires that MS collect relevant and comparable data on the volume of sales and on the use of antimicrobial medicinal products used in animals, to enable in particular the direct or indirect evaluation of the use of such products in food-producing animals at farms.

The Regulation includes a stepwise approach in which data from cattle, pigs, broiler and turkeys are to be collected from January 2024. From January 2027, data shall be collected for all food-producing animal species and from January 2030, data shall be collected for other animals which are bred or kept, which in practical terms adds to the data from the previously indicated food-producing species, all companion animals.

It is likely that the collection, and publication, of data on sales of antimicrobials, have helped countries to stimulate the activities to reduce AMC. Data collection (and publication) is one element more of AMR plans but it is to note that the press has published ESVAC data [234, 235], which is likely to have raised awareness of the need to reduce antimicrobial use in animals.

Overall it is encouraging to observe how some of the countries with some of the highest sales have reduced their sales of antimicrobials in mg/kg PCU, the new veterinary Regulation is likely to strengthen those reductions in all EU/EEA MSs.

10.3. HPCIAs.

In many countries cephalosporins can no longer be used in humans as empirical therapy due to AMR. Instead of those, carbapenems - an antibiotic class that represents the last available weapon against many gram-negative bacilli - are being used increasingly for empirical therapy [236].

As indicated on the ECDC/EFSA/EMA/SCENIHR joint opinion on antimicrobial resistance on zoonotic infections [237], the main mechanism of resistance to cephalosporins is through the production of β-lactamase enzymes which hydrolyse the β-lactam ring inactivating the cephalosporin. Resistance to antimicrobials in *Salmonella* and *Campylobacter* is of concern because antimicrobials may no longer be effective and treatment options be limited. In AMR *Salmonella* infections cephalosporins (which are used in children), may not be active and appropriate empirical therapy may be delayed.

A strong correlation has been found between ceftiofur-resistant *Salmonella enterica* serovar Heidelberg isolated from retail chicken and incidence of ceftiofur-resistant *Salmonella* serovar Heidelberg infections in humans across Canada [238]. This publication is the most relevant publication about the impact of reducing the use of antimicrobials in animals and reducing AMR

in humans [222]. According to Dutil *et al.* [238], changes of ceftiofur resistance in chicken *Salmonella* Heidelberg and *Escherichia coli* isolates appeared related to changing levels of ceftiofur use in hatcheries (before and after a voluntary withdrawal). According to the publication this provides evidence that ceftiofur use in chickens results in extended-spectrum cephalosporin resistance in bacteria from chicken and humans. The EMA/CVMP/CHMP AMEG scientific advice on the impact on public health and animal health of the use of antibiotics in animals [9] indicates that because of the importance ascribed to co-resistance in the horizontal transmission of resistance, decreasing the frequency of use of 3rd and 4th generation cephalosporins should be a high priority. The strongest evidence for potential beneficial effects to human health of risk mitigation measures involving reductions in the use of CIAs are from reductions in the occurrence of resistance to 3rd and 4th generation cephalosporins (and fluoroquinolones) in *E. coli* from broilers, poultry meat and pigs.

Thirteen countries decreased the sales of 3rd and 4th generation cephalosporins for use in animals between 2016 and 2010 (2010 or 2012), whilst 14 countries increased such sales.

Considering that 3rd and 4th generation cephalosporins are HPCIA according to the WHO [8, 155, 157-160], it is necessary to emphasize that the countries that have increased their sales should urgently review this situation in order to reverse it.

In the same manner that the number of countries that have increased the mg/kg PCU used of 3rd and 4th generation cephalosporins is of concern, the number of countries that have decreased, or practically stopped such use is encouraging.

The variation in sales of 3rd and 4th generation cephalosporins between the years 2010 to 2016 results in a not statistically different from zero increase of 13.7% of sales, which is not the result expected of such an important class of antimicrobials where a reduction on the use of cephalosporins in animals would be desirable.

As shown in Table 8, there is a high variation in % of decrease (or increase) of use of 3rd and 4th generation cephalosporins. From a decrease of -126% to an increase of 311.8% between the years 2010 and 2016, those high variations in a specific country might partially be caused by the small use in tonnes of those antimicrobials making that small changes in the use (or sales reported in one year) might result in a high variation in sales.

The high use of 3rd and 4th generation cephalosporins, and total antimicrobials in Cyprus, combined with a high overall antimicrobial sale, is noticeable and of concern from a public health point of view, especially considering the findings made in the country on AMR and AMC that concludes that very little has been done in Cyprus to the date of the report in the veterinary field to reduce the use of antimicrobials and encourage their prudent use. However, the same report indicates that is encouraging that the authorities are committed to implementing an AMR action plan [124].

The high use of cephalosporins with lower overall use of antimicrobials for Estonia and Luxembourg is noticeable and could partially be attributed to the use of cephalosporins in companions animals [87]. On the other hand, the low use of 3rd and 4th generation cephalosporins (zero or nearly zero) in Denmark, Finland, Iceland, the Netherlands, Norway and Sweden, with most of them with low overall sales of antimicrobials in mg/kg PCU is also remarkable, in some of those countries reduction of use of cephalosporins has been achieved by self-regulating the use of those HPCIA antimicrobials, like in Denmark where the poultry industry voluntarily stopped its use in 2002, the pig in 2010, and the cattle in 2014 which has resulted in a downward trend of resistance to cephalosporins in *Escherichia coli* isolated from broiler meat, pork and beef [125]. In Norway, 4th generation cephalosporins are not marketed whilst 3rd generation cephalosporins are only used in companion animals [131]. The latest available French report on AMC in animals

indicates that exposure of animals to newer-generation cephalosporins had decreased by 94.2% in 2017 compared to 2013, all species combined [40].

The 2017 WHO guidelines on the use of medically important antimicrobials in food-producing animals [135] make a series of recommendations in relation to CIAs. All the recommendations made by the WHO are to be applied for the 3rd and 4th generation cephalosporins including the complete restriction of use of all classes of medically important antimicrobials in food-producing animals for prevention of infectious diseases that have not yet been clinically diagnosed. The WHO also recommends that antimicrobials classified as critically important for human medicine should not be used for control of the dissemination of a clinically diagnosed infectious disease identified within a group of food-producing animals and finally that antimicrobials classified as HPCIA for human medicine should not be used for the treatment of food-producing animals with a clinically diagnosed infectious disease.

As already described in the introduction, the above-reported increase in the use of 3rd and 4th generation cephalosporins goes against the WHO and EMA/CVMP recommendations on the reduction of the use of CIAs.

The EMA/CVMP, in a document from 2009 [111], details recommendations that should be reflected in the sales of 3rd and 4th generation cephalosporins during the period analysed (2010 to 2016). Those recommendations indicate that 3rd and 4th generation (systemically administered broad-spectrum cephalosporins) are to be reserved for the treatment of clinical conditions which have responded poorly, or are expected to respond poorly, to more narrow-spectrum antimicrobials, and cautions the practitioners that increased use of those substances may increase the prevalence of bacteria resistant to cephalosporins. Those recommendations are aimed at reducing the use of cephalosporins in animals, and the onset of resistance generated by the use of cephalosporins due to its importance for human health. Liebana *et al*., [239] indicate that blaESBL and blaAmpC genes in Enterobacteriaceae are spread by plasmid-mediated integrons, insertion sequences, and transposons, some of which are homologous in bacteria from food animals, foods, and humans. These genes have been frequently identified in *Escherichia coli* and *Salmonella* from food animals. The publication notices that identification of risk factors for their occurrence in food animals is complex but that cephalosporin usage is an important risk factor for selection and spread of these genes. The publication highlights that there are no data on the effectiveness of individual control options in reducing public health risks, but that a highly effective option would be to stop or restrict cephalosporin usage in food animals, and that decreasing total antimicrobial use is also of high priority.

The EMA/CVMP document [111] also recommends that products for prophylactic use of systemically administered cephalosporins should always be limited to specific circumstances. The use of systemically administered cephalosporins for groups or flocks of animals such as the use of oral cephalosporins in feed or drinking water should be strongly discouraged. Those recommendations seem to be mild when compared with the EC regulation on veterinary medicinal products [1] which includes strong restrictions on prophylactic use of antimicrobials, or the WHO recommendations [135], which require a much more restrictive use of CIA antimicrobials. Use of 3rd and 4th generation cephalosporins in food-producing animals is mostly parental.

The OIE list of antimicrobial agents of veterinary importance [161, 162] indicates that amongst the VCIA in the OIE list, some are considered to be critically important both for human and animal health; this is currently the case for the 3rd and 4th generation of cephalosporins and fluoroquinolones. The mentioned list makes a series of recommendations for those substances including that they should not be used as a preventive treatment applied by feed or water in the absence of clinical signs in the animal(s) to be treated, nor to be used as a first-line treatment (unless

justified). It also recommends to urgently prohibit their use as growth promotors, which is not applicable to the EU/EEA that already banned the use of antimicrobial growth promoters years ago [42-45, 52, 240]. As clearly indicated on the WHO guidelines on use of medically important antimicrobials in food-producing animals [135], antimicrobial use in food-producing animals can lead to selection and dissemination of antimicrobial-resistant bacteria in food-producing animals, which can then be transmitted to humans via food and other transmission routes. The same guidance indicates that restriction of growth promotion use of antimicrobials in food-producing animals reduces the prevalence of antimicrobial resistance in bacteria isolated from food-producing animals that are, and can be, transmitted to humans. As described in this report (see chapter 5.12.5.), human patients with diseases caused by resistant bacteria are likely to have worse outcomes than those with the same disease without resistant bacteria.

From the above recommendations, most of which have been around since the start of the ESVAC data collection in 2010, it could have been expected to observe a reduction in the use of 3rd and 4th generation cephalosporins, whilst the analysis of data performed shows a (non-statistically significant) increase of sales.

Third and 4th generation cephalosporins are very potent antimicrobials (the amount required to treat animals is very small) whose use is very effective, and this might partially explain the increase in the average % of 3rd and 4th generation cephalosporins.

The latest ESVAC report [71] analyses the aggregated variation on sales of 3rd and 4th generation cephalosporins for 25 countries between 2011 and 2016, and finds a decrease in the sales from 0.25 (year 2011) to 0.21 (year 2016) mg/kg PCU, a decrease of 16% of the sales of 3rd and 4th generation cephalosporins.

Whilst it is good news that the aggregated sales of 3rd and 4th generation of cephalosporins have decreased, the increase in the comparison of the means of the 27 countries that had data from 2010 (2011 or 2012) to 2016 results in an increase of the average, which is of concern.na

Another analysed group of HPCIA are quinolones. Quinolones are known to select for quinolone-resistant *Salmonella* spp. and *E. coli* in animals. At the same time, quinolones are one of the few available therapies for serious *Salmonella* spp. and *E. coli* infections. Given the high incidence of human disease due to *Salmonella* spp. and *E. coli*, the absolute number of serious cases is substantial [160], which makes important to try to reduce the use of those substances in animals to those strictly requiring treatment. Three mechanisms for plasmid-mediated quinolone resistance have been discovered since 1998. The plasmid-mediated mechanisms provide only low-level resistance that by itself does not exceed the clinical breakpoint for susceptibility but nonetheless facilitates selection of higher-level resistance and makes pathogens containing those genes harder to treat [241]. Quinolones are used in human medicine for treatment of *Campylobacter* spp., *Salmonella* spp. invasive infection, MDR *Shigella* spp., *Pseudomonas aeruginosa*, *Streptococcus pneumoniae* and MDR tuberculosis [10].

According to the EMA/AMEG [10] quinolones (as of 3rd and 4th generation cephalosporins and polymyxins) are classified as category B (“Restrict”), which includes most of the WHO HPCIAs. The EMA/AMEG indicates that these restricted antimicrobials should only be used for the treatment of clinical conditions when there are no alternative antimicrobials in a lower category that could be effective. Especially for this category, use should be based on the results of AST, whenever possible.

In Table 10 it can be observed that, depending on the year, the lowest sales of quinolones per mg/kg PCU were for Sweden and Iceland, and the highest sales were for Italy, Hungary, Spain and Portugal. The biggest decrease in sales of quinolones sold per mg/kg PCU was for Iceland and the highest increase for Lithuania.

Fourteen countries had a decrease in their sales of quinolones, whilst 12 countries had an increase.

The decrease in the % of sales of quinolones in mg/kg PCU 2010 to 2016 is not statistically different from zero. The mean of the variation of sales of quinolones in mg/kg PCU shows a decrease of nearly -14%, which - even if not statistically different from zero - is encouraging, especially considering the many recommendations to reduce the use of quinolones [108, 109, 140, 155, 242, 243]. The latest French ANSES-ANMV report identifies an 87.8% decrease in exposure to fluoroquinolones in 2017 compared to 2013 [40].

Some countries have very small use of quinolones. In countries like Norway those are not used in the poultry industry [131], in Sweden - wherein 2016 only 14 out of 3,300 flocks of poultry were treated with antibiotics - no quinolones (polymyxins or cephalosporins) have been used in the poultry sector during recent years, on the Swedish dairy cattle sector only 0.3% of the milk infection cases where treated with quinolones [134]. Although in Sweden there are no special national legal provisions regarding the authorisation and distribution of CIAs, rules restricting their use in animals were introduced in 2013, these restrict the use of fluoroquinolones (and 3rd and 4th generation cephalosporins) to cases where a microbiological examination has been carried out, and susceptibility testing shows no other antimicrobials will be effective (with exceptions to e.g. treatment of acute life-threatening infections) [134].

Other countries like Belgium have also applied measures to reduce the use of quinolones. Since 2014 Belgium has imposed a sales tax on veterinary antibiotics, the tax is paid by marketing authorisation holders, the fee is €1.75 per kg of antibiotic active substance sold, and this is multiplied by 1.5 in the case of HPCIAs (fluoroquinolones, all cephalosporins and macrolides), the Belgium authorities on its 2014-2020 AMR plan indicate that they aim to lower by 75% the use of the most critical antibiotics (fluoroquinolones, 3rd and 4th generation cephalosporins) [223]. In the period of 2010 to 2016 use of quinolones was reduced by -60.0% in Belgium.

In Germany, there are strict guidelines with respect to the use of quinolones and cephalosporins, but no prohibitions on the use of a specific antibiotic [128]. In the period of 2011 to 2016 Germany had an increase of 12.1% in the use of quinolones.

In Denmark, veterinarians are only allowed to prescribe fluoroquinolones for a maximum treatment period of five days if an AST is carried out in a laboratory using an accredited method verifies that the agent causing the disease is not sensitive to other registered antimicrobials. In case of acute illness treatment with fluoroquinolones can be initiated before the result of the AST is available with the following conditions: The result of an AST carried out in the last eight days from a similar case in the herd is available, the AST confirms that the agent causing disease is not sensitive to other registered antimicrobials and the veterinarian can substantiate an acute illness history with high mortality in the herd, in addition within 14 days after finishing the treatment the veterinarian has to inform the authorities of the date of initiation and completion of the treatment, the number of animals treated, the diagnosis and result of the test and the details of the farm [125].

Whilst in countries like Romania prophylactic use of oral forms of fluoroquinolones in drinking water has been found, this is also partially due to its price as antimicrobial alternatives to fluoroquinoles might be more expensive [132]. In Cyprus where sales of quinolones have gone up substantially during the last years, officials from the Veterinary Services were not aware of the reasons for such increase [124].

In 2005 the FDA banned the use of fluoroquinolones in poultry because of the development of fluoroquinolone-resistant *Campylobacter* species in poultry which are transferred to humans and might cause the development of fluoroquinolone-resistant Campylobacter in humans and indicating

that fluoroquinolone-resistant *Campylobacter* infections in humans are a health hazard [244]. However, there are authorisations of fluoroquinolones for other animal species like pigs and cattle.

The latest ESVAC report [71] analyses the aggregated variation on sales of fluoroquinolones (excluding the other quinolones) for 25 countries between 2011 and 2016, and finds an increase in the sales from 2.54 (year 2011) to 2.70 (year 2016) mg/kg PCU, an increase of 6% of the sales of fluoroquinolones.

The overall sales of antimicrobials (expressed in mg/kg PCU) in 2016 are strongly correlated with the sales of quinolones in 2012 (0.657). The overall sales in 2010 are strongly correlated with the sales of quinolones during the same period of 2010 (0.664).

Quinolones are very important antimicrobials whose use is very effective, in animals and humans and that should be preserved in order to slow down the onset of AMR. Cheng, *et al.,* 2012 [245] indicate that Australia has restricted the use of quinolones in humans through its national pharmaceutical subsidy scheme and has not permitted the use of quinolones in food-producing animals. As a consequence, resistance to fluoroquinolones in the community has been slow to emerge and has remained at low levels in key pathogens, such as *Escherichia coli*. In contrast to policies in most other countries, this policy in Australia successfully preserved the utility of this class of antimicrobial drugs for the treatment of most infections. Although is not clear if the low resistance is the result of the actions in humans or in animals it suggests that limiting the use of quinolones might reduce the resistance to those important substances.

On those countries that have not reduced the use of quinolones there might be a need to further explain to users the necessity to reserve those important antimicrobials for very specific occasions when other alternatives have failed. In some countries the high number of MA of quinolones, and the low price of those substances might also have contributed to the lack of reduction of use.

Polymyxins (mostly colistin) have become the last resource antimicrobial in humans. Physicians in some countries have had to resort to antibiotics with unfavourable toxicity profiles and limited pharmacodynamic guidance like colistin due to the lack of therapeutic alternatives [236]. The WHO [160], indicates that polymyxins (e.g. colistin) are known to select for plasmid-mediated polymyxins-resistant *E. coli* in food animals. And that at the same time, intravenous polymyxins are one of few available therapies for serious Enterobacteriaceae and *Pseudomonas aeruginosa* multi-resistant infections in people in healthcare settings in many countries, especially in seriously ill patients in critical care. And that given the high incidence of human disease due to Enterobacteriaceae, the absolute number of serious cases where colistin is needed can be considered substantial.

The presence of a plasmid-mediated resistance mechanism (*mcr-1*), and other mcr genes, has left physicians with minimal alternatives in some life-threatening diseases like the treatment of patients infected with highly resistant bacteria such as carbapenem-resistant Enterobacteriaceae and *Acinetobacter spp.* for which other treatment options are limited, in addition in human therapy colistin is used topically by inhalation, especially in cystic fibrosis patients, as well as part of the regimen for selective decontamination of the digestive tract and of the oropharynx [186].

Since the discovery of the emergence of plasmid-mediated colistin resistance mechanism *mcr-1* in animals and humans in China in 2015 [184] many countries have taken action to reduce the use of antibiotics in animals, including the EMA recommendations to substantially reduce the use of colistin in the EU to levels below 5 mg/kg PCU or the desirable level of 1 mg/kg PCU [114]. The level of 5 mg/kg PCU was set based on limited data.

During 2016 eight of the 30 countries reporting data to ESVAC in 2016 were above the recommended 5 mg colistin per kg PCU, and 15 above the desirable 1mg colistin per kg PCU. It is

to note that the recommendations on polymyxins were made based on limits of overall AMC, i.e. not divided by specific animal species whilst ideally the recommendations should be focused in different animal species, to do so the AMC of colistin of each of the animal species should be know, this would also allow for the application of more refined indexes of AMC as the DDDvet or DCDvet [73, 77, 80].

As the EMA, CVMP and CHMP recommendation to reduce the use of colistin was delivered in 2016, the last date for which data were available for the preparation of this report, the recommendation should not have an important impact on the sales of colistin analysed. In any case, data available seems to indicate that even with limited time for implementation, there was a reduction on the use of colistin in many EU countries by 2016 and that such reduction has continued.

The mean of the sales of polymyxins in mg/kg PCU in 2016 (excluding countries without sales) was already 4.4 (see Table 14), i.e. below the recommended 5 mg/kg PCU. However, the level was recommended per country, so it is important that each EU/EEA country reaches the recommendation, not the average of all countries.

The decrease in sales of polymyxins in mg/kg PCU of 24 countries (excluding those without sales of antimicrobials) between 2010 and 2016 is -13.0%, although the decrease is not statistically different from zero.

As can be seen in Table 12, Figure 43 and Figure 44, 15 countries had a decrease in sales of polymyxins in the period 2010 to 2016, measured as mg/kg PCU, two countries had no variation, and 5 countries had an increase on those sales.

Three countries; Estonia, Netherlands and the United Kingdom had a remarkable decrease of 80% of their sales of polymyxins whilst Denmark had an increase 150%, however it is to be noted that the latest ESVAC report [71] indicates that sales of polymyxins are generally low in Denmark (0.5 mg/kg PCU in 2016) and that sales are expected to decrease in the future due to a new *yellow card* initiative according to which use of colistin gets an additional score when compared to the use of other antimicrobials [141, 142]. Sales of polymyxins in Denmark are already well below the EMA recommendations.

Since the discovery of the *mcr-1* gene many countries have taken active policies to decrease the use of polymyxins, including the recommended banning by the EMA/CVMP of the use of colistin associated with other antimicrobials [115, 116].

In the case of Sweden, a country that already has a low consumption of polymyxins, this has been applied by monitoring for the *mcr* genes in resistant isolates in the laboratory and in clinical isolates, as well as providing regularly updated information and raising awareness in scientific publications and in the press [134]. In Finland, systemically administered colistin is not currently authorised for use in animals [126].

The mg of polymyxins that are used in Spain per kg of estimated animal biomass (PCU) in 2015, is very high 34.9, this figure was reduced to 22.0 mg/kg PCU in 2016, this may already be due to some initiatives from the authorities and pig sector to correct such situation [123, 226]. A 3-year voluntary plan was initiated in 2016 in order to reduce the use of colistin [123], however the same report notes that several large operators met during the mission reported that they had until 2016 administered colistin in medicated feed to piglets at all stages of weaning, this has been confirmed by other sources [153]. The EC report also notes that substantial reductions in colistin use have been achieved with few negative and some positive effects, suggesting that much of the colistin use in the pig sector is either excessive or unnecessary.

In France, the EcoAntibio2 plan [246] set the goal of a 50% reduction in five years in exposure to colistin in the cattle, pig and poultry sectors, with a reference to the average exposure for 2014 - 2015.

Switzerland has reported that strong reduction in colistin sales might be linked to the introduction and extensive use of vaccines against both porcine circovirus and Lawsonia infections, thereby reducing the occurrence of diarrhoea and so the need to treat for bacterial secondary infections [71]. In some countries (e.g. Belgium, Denmark and France) zinc oxide had been used as a substitute for colistin [71, 125]. But due to concerns as to the use of zinc oxide on the environment (and also AMR related issues) the CVMP recommended the withdrawal of the existing marketing authorisations for veterinary medicinal products containing zinc oxide [248, 249], its use as a feed additive is allowed but at doses lower than those recommended for therapeutic use [250].

In line with the experience of some countries and the RONAFA report, vaccination, improved biosecurity, setting targets and avoiding routinely use of colistin in health programmes seem to be some of the actions conducive to the reduction of use of colistin,

Unfortunately, some countries did not show the same level of awareness as others on the need to reduce the use of colistin, e.g. some veterinarians in some countries seemed to be unaware of the concerns with the use of colistin [143, 144], is to note that at least one of those reports was produced around the time when the *mcr*-1 gene was found and that awareness of the problems linked to the use of colistin is now likely to be higher.

The EMA recommendations on colistin have become a clear example of how the use of data on antimicrobial sales allows for a rational recommendation on the use of an antimicrobial (colistin). While allowing the substance to remain authorised for use in animals, it does impose strict recommendations for limiting its use. If the ESVAC data would not be available the finding of the *mcr-1* - and related mcr genes, could have resulted in the prohibition of the substance in animals in the EU/EEA, the results of the action might determine in the future if similar actions at EU level can be taken in which based on AMC data, specific risk mitigation measures, as targets, can be established, is now to be seen if the recommendations to reduce use of colistin does not result in an increase of use of other substances like amynoglycosides.

As shown in Table 30 and Table 31, there is a very strong significant Pearson's linear correlation (0.861) between the sales of antimicrobials in mg/kg of PCU 2016 and the sales of polymyxins in mg/kg PCU during the year 2016, as indicated above, this correlation is also found with the sales of quinolones (0.792, year 2010 and 0.693 year 2016) and the total sales in tonnes of antimicrobials (0.836 year 2010 and 0.773 year 2016, see Table 31), this seems to suggest that the fewer antimicrobials a MS uses, the fewer polymyxins will be used.

Most of the use of colistin is oral in pigs and poultry [251], with some use in other species like veal cattle [112], there is not a correlation between the use of those substances and the % of pigs and poultry. This is probably because other factors like the tradition of use of colistin in a country, or the availability of authorised products are also of relevance.

There is a moderate significant Pearson correlation between the % of pigs and the variation of sales of polymyxins between 2010 to 2016 (not at the cut-off point of the Bonferroni correction) of 0.463 (see Table 32), which could suggest a moderate correlation between the % of pig population in a country and the decrease (or increase) of sales of those substances.

A strong correlation can be found between some of the sales of group treatment and the sales of polymyxins (colistin) in 2016: the % of premixes (0.614), and the sales of oral solution (0.734) are strongly correlated with the sales of polymyxins (both cases at the cut-off point of the Bonferroni correction, see Table 31), which confirms that colistin is mostly used in oral group treatments.

Following the finding of the *mcr-1* gen in China, The Lancet published in 2016 a comment about the banning of colistin as growth promoter in China [252], in which it was indicated that from 1st November 2016, colistin was no longer authorised as a growth promoter in this country (whilst at the same time disagreeing with the EMA considerations on the vertical transmission of colistin resistance genes). Remarkably the same publication indicates that more than 8,000 tonnes of colistin would not be used in the veterinary sector as a result of the measure, it is not apparent if the use of colistin as a medicinal product has now been banned or not, but there are two main remarks to this action;

- Antimicrobial growth promoters are not allowed for use in the EU since 2006 [41, 240], and the authorisation of use of an antimicrobial classified as HPCIA by the WHO [155] as AGP should completely restricted as recommended by the WHO [135].
- The total amount of antimicrobials that will not be used according to the publication is 8,000 tonnes, and the total amount of antimicrobials in all the countries reporting to ESVAC in 2016 was 7,787 tonnes (total use of polymyxins was 397 tonnes). It is quite remarkable that the amount of use of a HPCIA is about the same amount of the total of antimicrobials reported to ESVAC by 30 EU/EEA countries.

The latest ESVAC report [71] analyses the aggregated variation on sales of polymyxins for 25 countries between 2011 and 2016, and finds a decrease of sales in mg/kg PCU from 11.01 (year 2011) to 6.62 (year 2016), a decrease of 40% of the sales of polymyxins. Is interesting to observe how the aggregated method of calculation (see 9.1.2.) provides a strong reduction of sales of polymyxins than the method used in this report in which the average of sales of polymyxins in mg/kg PCU of each country is divided by the number of countries, this shows that the high sales in a few countries can strongly impact the average use of the substance in the EU/EEA.

The first conclusion that can be reached is that there is an overall decrease in the sales of antimicrobials between 2010 and 2016, the second is that this reduction is not replicated (or statistically significant) for 3rd and 4th generation cephalosporins, quinolones and polymyxins.

These data suggest that there has been a focus into overall antimicrobial reduction but that this focus might not have had the desired impact into some of the HPCIA and that it would be advisable to consider not only the overall consumption of antimicrobials but also other classes of antimicrobials, and more specifically, 3rd and 4th generation cephalosporins, quinolones and polymyxins.

10.4. Pharmaceutical forms

The oral group medication pharmaceutical forms (e.g. premixes) in which the antimicrobials are administered to the animals is one of the main differences in respect to the use of antimicrobials in humans. In animals, in addition to individual administration like injections or tablets there are other group medication forms, e.g. medicated feed (a homogeneous mixture of feed and veterinary medicinal products), "*top dressing*" (manual mixing of a veterinary medicinal product) or water for drinking with a veterinary medicinal product (oral solution).

The reason for the above-described administration of VMPs to animals is that animals for food production are grown in big herds where in case of disease they are treated as a group. In many cases treatments are preventive to avoid dissemination of diseases. There are also specific cases like treatment of mastitis, a disease which is the biggest reason for use of antimicrobials in dairy cows. Other specific treatments are administration of antimicrobials in the milk in e.g. cases of respiratory diseases of calves [208]. For pigs respiratory disease and diarrhoea in weaning pigs are

the most often indications for antimicrobials [253], those are usually treated by oral medication or individual injection of the whole group.

As exemplified above, antimicrobials can be provided in many pharmaceutical forms to animals. The ESVAC project has simplified those forms into premix, oral powder, oral solution, injection, oral paste, bolus, intramammary preparations, intrauterine preparations and tablets.

Oral use of antimicrobials, especially low dosages of oral prophylactic and therapeutic group medication, might convert not only the commensal microbiota from the digestive tract but also the opportunistic pathogenic bacteria in the respiratory tract into reservoirs of multi-resistance [254], which makes the high use of oral formulations for group medication especially worrisome for two reasons:

- The less targeted treatment of the animals when compared with individual treatments.
- The exposure of the gastrointestinal commensal flora to the antimicrobials and consequent possibility of selection for resistance.

The leftovers of antimicrobials of oral group treatments in e.g. drinking water are also of concern.

The EMA/EFSA RONAFA opinion [139] indicates that oral administration is of particular concern in terms of promoting the development of AMR due to the high exposure of gastrointestinal commensal bacteria, and the sometimes prolonged duration of treatment/exposure. Parenteral administration may also expose the intestinal microbiota if the antimicrobial is excreted in an active form into the gut lumen. As noted by the mentioned opinion and confirmed by the data in Table **17**, there is an important variation in sales in the EU/EEA MSs in the proportions of premixes, oral powders and oral solutions sold. The RONAFA opinion suggests that this may reflect species distributions and national policies, but others have also indicated that there is no clear reasoning for such differences [255].

Dupont *et al.*, 2017 [141] in a study analysing the impact of the yellow card in pig herds in Denmark concluded that less use of group medication was one of the driving factors for reducing AMC.

Medicated feed is most commonly used in intensive production [139], especially of pigs, but there is an unequal use of medicated feed depending on the country. Some countries use little medicated feed (see Table 17) whilst other alternatives such as oral powders or oral solutions are used. The reasons why some countries favour one or the other pharmaceutical form cannot be analysed in detail here but technological reasons, national regulations on medicated feed or availability of authorised medicinal products might be part of the reason why there is such a variety of % of administration of the different pharmaceutical forms for oral group treatment.

In an EC commissioned report on medicated feed [255] it is indicated that there is no generally valid economic rationale for farmers to prefer a specific way of administering oral veterinary medicines, be it through medicated feed or water medication. Whether medicated feed is a more costly or a more cost-efficient alternative of administering oral VMPs compared to water medication depends on the pricing strategy applied by manufacturers of medicated feed, the active substance used and the specific Member State, so no overall conclusion can be made on why some MSs prefer one method of administration towards the others. Is also noted that the main advantage of medicated feed is that it ensures homogeneity and stability of the VMP in the feed and reduces the number of people handling highly concentrated veterinary medicines.

Of those pharmaceutical forms, the premix, oral-powder and oral solutions are most likely used for group treatment. Injectable forms will also be used for groups of animals, but injectables have to be administered individually which makes it more laborious for the mass treatment of animals

(although might be a common practice in piglets and feedlots). The same is true for the other pharmaceutical forms; oral paste, bolus, intramammary and intrauterine preparations.

The EMA/AMEG revised categorisation [10] indicates that relatively little attention has been given to the association between the antimicrobial formulation (pharmaceutical form) and the rise of multidrug-resistant (MDR) organisms. It highlights that administration of antimicrobial agents through either bulk animal feed or the drinking water supply, rather than by injection, has major economic and ergonomic advantages, but also avoids some of the potential unwanted effects of injections such as carcass damage or residues at an injection site. The advice notes that in some situations (e.g. commercial chicken production, aquaculture) oral administration to the whole group of animals is almost always the only feasible option (however there also some treatments like vaccines provided individually to those animals). Interestingly the document also raises that the withdrawal time is in general longer for VMPs administered by injection compared to VMPs administered orally.

The EMA/AMEG categorisation also highlights that for orally administered antimicrobials there are several opportunities for the incorrect intake of dose and for the antimicrobial to present an AMR selection pressure before the agent reaches the target tissue at a concentration able to inhibit or kill the micro-organism involved in an infection. The categorisation, making reference to other publications, indicates that in in-feed medication, the adequate mixing and homogenous distribution of the antimicrobial rely on factors like the particle size and electrostatic properties of the premix, as well as the final composition of the feed and the mixing equipment used. And that the same equipment may also be used for the production, storage and/or transport of both medicated and unmedicated feed, with the potential carry-over of antimicrobial residues, and that oral administration via drinking water can be more precisely dosed compared to medication administered in food. Although not reflected in the AMEG report, the time required for the delivery of medicated feed once a prescription from the veterinarian is provided might also be one of the relevant factors when deciding the pharmaceutical form of administration of the antimicrobials to the animals.

Other factors contributing to variable intake of oral group medications include a relatively poor control over intake due to hierarchy in the flock/group, a lower intake by diseased animals, uncertain duration of therapy and potential for cross-contamination of feed.

The AMEG revised draft advice also addresses how the selection of AMR may depend strongly on the pharmaceutical form and how, as an example, certain antimicrobials administered parenterally can be actively excreted in the gut, via bile, where a selection pressure for AMR can be expected.

Finally, the draft revised advice [10] provides a suggested listing of routes of administration and formulations, ranked in order from those with general lower effect on the selection of AMR to those that would be expected to have a higher impact on resistance, as follows:

- Local individual treatment (e.g. udder injector, eye or ear drops);
- Parenteral individual treatment (intravenously, intramuscularly, subcutaneously);
- Oral individual treatment (tablets, oral bolus);
- Injectable group medication (metaphylaxis), only if appropriately justified;
- Oral group medication via drinking water/milk replacer (metaphylaxis), only if appropriately justified.
- Oral medication via feed.

To conclude on this subject, the AMEG advice notes that the above information is based on a simple review of the literature and that the conclusions drawn and the proposed order of ranking should be confirmed by a systematic review followed by a meta-analysis in which clinical efficacy and microbiological impacts should be studied as outcomes.

It is essential that antimicrobials provided via feed or water are properly homogenised, so animals will receive the required amount of those and some will not be overdosed, it is also to be noted that some animals may continue to drink after they have stopped feeding [139].

As done by the ESVAC project, tablets are excluded from the calculations as those are likely to be given to companion animals which are not included in the animal biomass (or Population Correction Unit) [71].

The % of oral forms for group treatment was calculated by adding the 3 main forms administered as group treatment i.e. premix, oral powder and oral solution. The forms of oral paste, bolus and tablets were disregarded from this calculation as although they are administered orally; they are administered individually to the animals. When discussing the oral forms for group treatment in this chapter, it refers to medicated feed, oral powders and oral solutions.

The biggest increase in the % of oral forms for group treatment use between 2010 and 2016 is in Lithuania (20.8%), whilst the biggest decrease is in the United Kingdom (-13.1%), no further conclusion could be drawn from this especially since the variation in % of oral forms from the years 2016 to 2010 (2011 or 2012) is not statistically different from zero ($p = 0.570$, 2-tailed).

Cyprus is the country that uses the most premixes in mg/kg PCU (358.7), followed by Spain (248.2) (see Table 16). Eleven countries (Denmark, Estonia, Germany, Iceland, Latvia, Lithuania, Luxembourg, Netherlands, Norway, Slovenia and Sweden) have sales of premixes below 1 mg/kg PCU.

Some of the countries with low % of premixes (0.1%, see Table 17), would have high sales of other oral forms (oral powders or solutions), like Germany where most of the sales in 2016 are for oral powders (47.1%) which raises questions about the homogeneity of the use of those substances, or the distribution of oral solutions (44.1%) (see Table 17) [139].

A relatively similar case of low sales of premixes would be Lithuania which has a high/medium % of oral forms (70%), low sales of medicated feed (0.5%) but a high % of oral powders (53.9%) and solutions (15.6%), or Estonia where the % of products for oral administration (69.1%) (nearly the same as in Lithuania) and the sales of medicated feed is practically zero percent and most of their antimicrobials are sold as oral powders (66.8%) or as oral solution (2.2%).

A different case would be Cyprus that has a high % of products for oral administration (95.6%), of which most is medicated feed (79.1%), whilst having relatively small sales of oral powders (12.9%) or oral solutions (3.6%).

The Belgium authorities have produced ambitious plans to reduce AMR, in humans and animals which includes halving the use of medicated premixes containing antibiotics by 2017, which was achieved by a 69.8% in 2018 [221, 223]. The main objectives in respect to the use of antibiotics reducing by half the overall use of antibiotics by year 2020 (in respect to year 2011), of which a 35.4% seems to have been already achieved by 2018. The plan also aims to, reduce by 75% the use of most critical antibiotics also by 2020; which has already been achieved by 79.1% in 2018 [223].

As can be observed in Table 15, seven countries have a percentage of sales for group treatment that are between 100 and 90% (Hungary, Spain, Cyprus, Portugal, Italy, Bulgaria and Germany), nine for which the % is between 90 and 80% (Belgium, Poland, Greece, Romania, Czech Republic,

Austria, Netherlands, Slovakia and France). The countries with the lowest sales in the % of oral forms for group treatment are Sweden (10.8%), Norway (9.3%) and Iceland (4.5%).

The above variety of percentages of use of oral forms of antimicrobials confirms that different MSs have followed different paths in relation to the pharmaceutical forms used for the treatment of animals.

There is a strong Pearson's linear correlation (within the Bonferroni cut-off limit) between the % of oral forms in 2016 with the overall sales in mg/kg PCU in 2010 (0.655), and a moderate correlation of the same % with the overall sales in 2016 (0.585), which shows that there is a relationship between the % of oral forms with the overall sales of antimicrobials, this was to be expected as many mission fact-finding reports from the EC confirms such relation [32].

For the years 2010 to 2016 sales of oral forms decreased in a remarkable manner for Italy and Germany, countries that have notably reduced their antimicrobial overall use (-30.0% and -57.8% respectively, see Table 4).

For two countries the % of premixes are above 65% in 2016: Cyprus (79.1%) and Spain (68.5%). For 8 countries the % of sales of premixes are equal to or below 1% (Slovenia, Netherlands, Lithuania, Iceland, Germany, Luxembourg, Latvia and Estonia).

The Pearson's linear correlation between the sales of premix (in mg/kg PCU) in 2016 and the overall sales in mg/kg PCU 2016 is very strong (0.944).

The correlation of the % of premixes (as a proportion of all pharmaceutical forms) *vs* the overall sales of antimicrobials in 2016 also have a high correlation (0.716) (within the Bonferroni cut-off limit).

Those correlations show that countries with high overall sales of antimicrobials have a high use of premixes (as total sales, or as a % of the total), and the opposite, i.e. that countries with small sales of antimicrobials have small sales/proportion of premixes.

The above correlations seem to suggest that reducing the use of premixes (or other forms of oral group medication) in a country might be one of the most useful measures to reduce overall AMC.

Regulation 2019/4 [152] on the manufacture of medicated feed, includes many measures intended to fight against AMR, by e.g. not allowing the use of medicated feed for prophylaxis and allowing metaphylaxis only in cases of high risk of spread of diseases.

Table 18 shows how the three countries that have the highest % of individual treatments (and correspondingly the lowest % of sales of oral forms for group treatment) are also those with the lowest sales of antimicrobials expressed as mg/kg PCU. Whilst some of the countries with high % of sales of oral forms for group treatment are also some of those with high sales of antimicrobials expressed as mg/kg PCU (e.g. Cyprus, Spain and Italy), the highest % of sales as oral sales per group treatment does not correspond to the country with the highest sales of antimicrobials in mg/kg PCU (i.e. Hungary). It could be speculated that oral group treatments are less effective in treatment than individual treatments as the dose might not be equally distributed between animals due to e.g. health status of the animals, or other factors like hierarchy. This could result in higher amount of antimicrobials required to treat animals when compared with individual treatments.

In any case, all the countries that are included in the group of countries with the highest consumption of antimicrobials as mg/kg PCU (see Figure 15) (Cyprus, Spain, Italy, Portugal, Hungary, Bulgaria, Belgium and Poland) have a % of sales for oral group treatment above 88%.

The report of the EC fact-finding mission carried out in Spain in October 2016 to gather information on the prudent use of antimicrobials in animals indicates that in several cases, colistin, amoxicillin

and oxytetracycline were administered in the medicated feed as preventive treatments for diarrhoea, meningitis or respiratory conditions. In several other cases, doxycycline, tylosin and colistin were administered in medicated feed for reasons given as initiation of fattening. The report also highlights that one of the operators interviewed noted that piglets at weaning are systematically treated with amoxicillin in medicated feed and, prior to this, colistin was included in all three types of feed given to piglets during the weaning stage. Antimicrobial pig preventive treatment has been confirmed also by other sources, not only at weaning but also at finishing stages [123, 153, 251, 256].

Figure 53. Sales in mg/kg PCU in 2016 vs percentage of oral forms for group treatment in 2016, shows how the linear fit is not the best to express the sales of antimicrobials *vs* the % of oral forms for group treatment. For those cases with the lowest sales of antimicrobials expressed as mg/kg PCU and with the lower % of oral pharmaceutical forms for group treatment (Iceland, Norway and Sweden), the linear fit is not suitable, whilst for those countries with higher % of oral forms for groups treatment (upper right side of the graph), is very visible how Cyprus, Spain and Italy are far away from the linear fit.

The % of pigs plus poultry production in Iceland and Norway is 9.5% and 10.4% (see Table 25), which could partially explain the low sales of antimicrobials in those countries. The Pearson's linear correlation with the overall sales of antimicrobials in 2010 or 2016 is moderate, 0.481 and 0.439 respectively and its significance is below the Bonferroni correction (see Table 32 and Table 36).

Figure 54. Sales in mg/kg PCU in 2016 vs percentage of oral forms for group treatment in 2016, with a non-linear fit (Loess fit**)**, provides a much better fit of the data showing a very interesting, nearly exponential, fit of the graph. The exponential relationship is 0.835 between both indicators, significant and fits better than a linear one (0.339) (Figure 55).

This fit seems to suggest:

- Countries with low sales of antimicrobials expressed as mg/kg PCU have a very low % of sales of antimicrobials as oral group treatment, this goes beyond the linear fit and seems to indicate that if the oral sales are very low the overall AMC will be even lower. Denmark has medium sales of antimicrobials for oral group treatment (60.6%) and high pig production but has maintained a low overall consumption of antimicrobials (40.8 mg/kg PCU in 2016).
- Countries with high sales of antimicrobials expressed as mg/kg PUC have a very high % of sales of antimicrobials as oral group treatment, this goes beyond the linear fit and seems to indicate that if the oral sales are very high the overall AMC will be really high. In other words, only by using oral group treatments it is possible to reach the highest figures of AMC in mg/kg PCU.

The relation between the indicator "sales of antimicrobials expressed as mg/kg PCU" and "% of oral sales for group treatment" is not linear but exponential.

The fit line in the correlation of the % of injectables *vs* total sales in 2016 (Figure 67) shows a clear tendency to decrease the mg/kg PCU as the % of injectables decreases. The significant exponential correlation seems a better fit for the graph than the linear fit (Figure 68).

As can be observed in Table 17, for three countries (Iceland, Sweden and Norway) the sales of injectables are above 60% in 2016. For nine countries the sales of injectables in 2016 were below 10%: Hungary, Cyprus, Spain, Portugal, Italy, Bulgaria, Germany, Poland and Belgium.

For three countries (Iceland, Norway and Sweden) the sales of individual forms were above 80%. For nine countries sales of individual treatments in 2016 were below 10%: Hungary, Spain, Cyprus, Portugal, Italy, Bulgaria, Germany, Poland and Belgium.

The above percentages indicate a very high variation in the use of different pharmaceutical forms of antimicrobials in the EU/EEA countries. When considering that those countries with high injectable or individual treatments are low on the overall consumption of antimicrobials and vice versa, it suggests that increasing the % of the use of individual treatments like injectables versus pharmaceutical forms for oral group treatment of antimicrobials could be one of the measures to reduce antimicrobial use.

The fit line in the correlation between the % of individual forms *vs* total sales in 2016 (Figure 71) shows a clear tendency to decrease the sales of antimicrobials expressed as mg/kg PCU as the % of individual treatment increases. The significant exponential correlation better fits the graph than the linear fit (Figure 72).

There is hope that the implementation of the Regulation 2019/6 on VMPs [1] and the Regulation 2019/4 on medicated feed [152] will provide the necessary tools to reduce the prophylactic use of antimicrobials in groups of animals. Ideally, the WHO [135], OIE [162], FAO [37] and OECD [257] recommendations on prudent use of antimicrobials in animals should bring the same results on reduction of AMC worldwide as otherwise there is the risk that the reduction of AMC, and of AMR, will only be focalised in the EU/EEA and not provide the desirable AMR reduction at a global level.

10.5. Collecting data per animal species

The collection of AMC data per animal species and its possible link to the amount of antimicrobial sales in mg/kg PCU was analysed. The reason for such analysis is to identify if the action of collecting data per animal species is linked to reductions in the sales of antimicrobials for animal use at a country level.

Collecting data per animal species has many added values in respect to the more rough collection of sales data, including the ability to identify the animal sectors that can benefit the most with prudent use of antimicrobials policy, and better targeting the antimicrobials for which consumption reduction should be most desirable, namely those classified as HPCIA by the WHO [160].

The criteria of which countries were categorised as collecting data by animal species was the criteria set by the "network on quantification of veterinary antimicrobial usage at herd level and analysis, communication and benchmarking to improve responsible usage" (see 9.4. for further details).

The mean of the sales of antimicrobials (mg/kg PCU) in 2016 of those countries collecting data by animal species is 89.1, the mean of those not collecting those data by animal species is 108.2 (see Table 20).

The comparison of the sales of antimicrobials (mg/kg PCU) in 2016, depending on collecting data by animal species, using the Mann-Whitney U test, does not allow concluding differences between those two groups of countries ($p = 0.539$, 2-tailed).

In addition, to analysing the sales of antimicrobials in mg/kg PCU for the year 2016, the variation in mg/kg PCU for the years 2010 to 2016 *vs* the collection of data by animal species in 2016 was also analysed.

In the period 2010 to 2016, the 15 countries collecting data per animal species have a higher reduction of AMC (-26.6%) versus the 12 countries not collecting data by animal species (-5.3%). However, the comparison of the variation of AMC (mg/kg PCU) between the years 2010 to 2016 depending on collecting data by animal species, using the Mann-Whitney U test, does not allow concluding differences between them (p = 0.059, 2-tailed).

The lack of conclusive results is surprising as it could be expected that those countries that collect data by animal species have a lower AMC as the collection of data by animal species results in better control of the use (not only sales of antimicrobials) and should result in lower overall consumption. However, a detailed analysis of the countries that are collecting data by animal species might help to explain the lack of significance of the results.

As an example, one of the countries with the lowest AMC in mg/kg in PCU in 2016 (Iceland, 4.7) does not collect data by animal species. Iceland has a small animal population and the husbandry system, as well as the responsible use by veterinarians, does explain its low use of antimicrobials.

On the other hand, some of the countries with the highest AMC in mg/kg PCU, e.g. Spain (362.5) and Italy (294.8) do have (partial or under implementation) systems to collect data on AMC [123, 145], the impact of those systems on the overall data collection seems, however, to be limited. The reasons are not clear, but it might be because there is not yet a publication of the results at a national level, or because data collection has only been implemented in some production types or parts of the country. The decrease in AMC could be expected once those data at national level are made publicly available, and nationally comprehensive benchmark systems are in place that encourages animal producers to reduce AMC.

In addition, some of the countries that have set a benchmarking system seem to have obtained the biggest reduction in AMC (see Table 4), e.g. Germany (-54%) and the Netherlands (-56%). The other country with the biggest AMC reduction, France (-48%) has not set up benchmarking systems but has taken many initiatives by sector, including setting overall targets of AMC reduction and by some HPCIAs and increasing awareness at the country on the need to reduce AMC [59, 229].

As described above, the New Veterinary Regulation 2019/6 [1] will oblige the EU MS to collect data per animal species which will provide an invaluable set of data to assess if the collection of data by animal species might result in an overall reduction of AMC, and eventually AMR.

The EU/EEA countries are likely to implement different systems of data collection, and it will be of interest to analyse, which of those result in a more effective reduction of AMC.

10.6. Collecting data before 2007 (pre-ESVAC) and sales of antimicrobials in 2016.

In 2016, the 10 countries collecting data on AMC in animals before 2007 have a much lower consumption in mg/kg PCU (44.1) versus the 20 countries not collecting data by 2007 (125.9).

Non-parametric two independent test (Mann-Whitney Test) were applied, concluding that the differences between both groups are significant (p = 0.002, 2-tailed).

This is not surprising as it could be expected that the countries that started the collection of sales data earlier would have a keen interest in the reduction of antimicrobial use in animals.

In the period 2010 to 2016, the 10 countries collecting data on AMC in animals before 2007 have a higher reduction of AMC (-36.1%) versus the 10 countries not collecting data by 2007 (-5.9%).

The Mann-Whitney test allows us to conclude that the variation in sales in both groups is statistically different (p = 0.006, 2-tailed).

This continued reduction in the sales of antimicrobials in countries collecting data before 2007 (-36.1%) is remarkable. Most of the countries that were collecting data before 2007 already had a low AMC by 2010. Those countries that were not collecting AMC data by 2007 had more room for rapid antimicrobial use reduction by e.g. reducing the routine prophylactic use of antimicrobials.

The findings of the EC missions on prudent use of antimicrobials seem to provide confirmation that the countries that were involved in the collection of data on AMC before 2007 have continued with those activities which have led to a further decrease of sales of antimicrobials [125, 128, 130, 131, 134], whilst other countries that have only recently started to collect data on AMC have had a much more limited activities on reducing AMC [32].

Some countries like Sweden have been collecting data on AMC in animals for a long time. The latest Swedish report on AMC and AMR reports the sales of data since 1980 to 2018 an amazing period of 38 years [258]. The latest Danish report on AMC provides sales from 1990 [54]. The Norwegian report reports sales data since 1993 [259]. All those years of data collection and activities to reduce AMC seem to have been effective to implement prudent use policies and reduce resistance, as well as increasing awareness of the problem of AMR.

It could be argued that for the collection (and publication) of data on AMC to result in a decrease in sales of antimicrobials, there is a need for some years to pass. Although this provides a pessimistic view on the need for years to reduce AMC, there is also a positive side to this view; years after the publication of sales data (which the author believes triggers activities to reduce AMC), the reduction on AMC can be long-lasting.

10.7. The animal species produced is associated with the overall sales of antimicrobials

Different animal species will have different requirements for antimicrobial use due to the animal species and the production systems. The animal composition in different countries *vs* the AMC was studied.

Some of the most antibiotic hungry species (pigs and poultry [179, 208]) were added as a proxy of the intensive animal's production in a country. This addition intends to estimate the animal categories that are most likely to be produced in intensive conditions.

The FAO report on tackling antimicrobial use and resistance in pig production in Denmark [206] indicates that specialization in the Danish pig production sector has meant that in 2018 there are fewer than ten specialized pig practices providing veterinary advisory services to virtually all pig farmers in the country, given them an important role on AMC reduction.

Species like sheep or goats tend to be treated with fewer antimicrobials when grown in extensive conditions [208].

The analysis of the % of the PCU for the sum of pigs and poultry, or for pigs, poultry or cattle separately as a proxy for intensive production is very rough. Species like cattle (especially those produced to be sacrificed at an early age) are produced in many cases in an intensive manner with high consumption of antimicrobials [89, 208], there is however no denying that the method of production and the species produced does impact the overall AMC of a country.

The variation on the animal production in different countries is remarkable; this makes it even more important that in the future data are collected by animal species [21, 26, 74, 86]. As an example, the mean of the % of the combined sales of pigs and poultry is 41.7% with a minimum of 9.5%

(Iceland) and a maximum of 78.3% (Denmark, see Table 25), interestingly both countries are some of those with the lower sales of antimicrobials for animal consumption in 2016 (see Table 4).

None of the Pearson's linear correlation of the % of pigs, poultry, cattle, caprinae or the combination of pigs and poultry is statistically significant when analysed *vs* the sales of total antimicrobials in mg/kg PCU, 3rd and 4th generation cephalosporins or polymyxins (within the Bonferroni cut-off limit, see Table 32 and Table 33). Only the % of poultry in 2016 was moderately correlated with the sales of quinolones in 2016 (0.570) within the Bonferroni cut-off limit. Other correlations were identified, e.g. the moderate correlation between the sales of overall sales of antimicrobials in 2016 and the % of pigs and poultry in 2016 (0.439), but those were below the Bonferroni cut-off limit.

The limited number of significant correlations between sales of antimicrobials and animal species was not expected, as more correlations with different animal production species could be expected. As noted above this might be the result of the proxy (% of PCU of an animal species in a country) not been sufficiently detailed to allow for stronger correlations between animal species and AMC.

10.8. The average temperature in a country is correlated with the overall use of antimicrobials in animals in the country

The influence of temperature and rain (average precipitation) on the overall AMC has been studied to find its possible correlation with the sales of antimicrobials, the results in mg/kg PCU of antimicrobials sold during the year 2016 were used as the reference.

MacFadden, D.R., *et al*., 2018 [205] reported that recent emergences of highly mobile genetic elements of resistance have originated from central latitudes (areas of higher temperature) and that increased gene transfer might then be expected to facilitate population transmission. The publication indicates that temperature is one of the most potent modifiers of bacterial growth rates and may drive increased carriage and transmission of resistant strains between humans and animals. I.e. driving the environmental growth of resistant strains and leading to increased transmission of resistance from food, agriculture and environmental sources; the publication suggests that this could lead to a higher need to use antimicrobials in more warm areas, especially on animals that can be argued that are more in contact with the surrounding environment than humans.

The author's claim that the potential temperature associations may be rooted in more complex factors (for example, behavioural and social) that occur across humans, animals, agriculture and environment. All those factors provide a plausible explanation to the idea, expressed in an informal manner to the author of this report by, e.g. animal producers or veterinarians, that the warmer the temperature, the more presence of resistant bacteria, and behavioural and social factors, that promote more use antimicrobials.

The Pearson's linear correlation of the average temperature (ºC) *vs* the overall sales in mg/kg PCU 2010 is a very strong correlation (0.812). The Pearson's linear correlation of the average temperature (ºC) *vs* the overall sales in mg/kg PCU in 2016 is a strong correlation (0.768) (see Table 32).

Similar correlations were found with the average high temperature and the average low temperature that was correlated with the overall sales in mg/kg PCU in 2010 and 2016 (see Table 32 and Table 36), the results were also of very strong or strong correlations.

The Pearson's correlation of the average, high and low temperature, with the sales of polymyxins in 2010 or 2016 is strong in a range from 0.622 to 0.684 (see Table 32 and Table 36), which suggests that use of certain types of antimicrobials might be correlated to the temperature in the country.

An estimation was also made with the average precipitation (mm) versus the sales in mg/kg PCU in 2016 which was moderate (-0.411). Although the correlation is significant at the 0.05 level, it was not within the Bonferroni cut-off limit of 0.00147.

There are too many variables to attribute causality between the use of antimicrobials to animals to temperature. However, it is an area that merits further exploration.

The variation of the use of antimicrobials with temperature could be motivated for various reasons, it could be speculated that the presence of more resistant bacteria in the environment in which the animals are grown, could result in the need to use more antimicrobials in animals, but could also be linked to, e.g. cultural attitudes linked to temperature.

The found correlations make even more critical for those countries with high temperature to promote more stringent policies on AMC reduction as:

- Those countries are more likely to use more antibiotics (see Table 32 and Table 36).
- May drive increased carriage and transmission of resistant strains between humans and animals [205].

In any case, to use temperature as an excuse for high AMC is a poor reason that should be avoided.

Conclusions

11. Conclusions

1. The data analysed show that EU/EEA and Switzerland countries have a very different AMC, with high differences on antimicrobials consumed per kg of animal produced.
2. The data available suggests that to reduce AMR there is a need to implement policies at the country level that reduce AMC, especially in those countries with high and medium consumption of antimicrobials. In the EU/EEA there are plenty of good examples on how to efficiently reduce AMC, including implementation of prudent use guidelines and setting targets on AMC.
3. Some studies suggest that the reduction of AMC in animals might result in the reduction of AMR. Those reductions on AMC are the result of implementation of national policies and recommendations by the EC, WHO and OIE as well as the implementations of surveillance projects like the ESVAC.
4. A reduction in antimicrobial use in animals could reduce exposure of the environment to antimicrobials and also reduce exposure of animals and humans, to those antimicrobials.
5. In the period 2010 to 2016 many countries in the EU/EEA have reduced overall antimicrobial use in animals (expressed as mg/kg PCU). The results of sales of antimicrobials in mg/kg PCU indicate that the decrease in sales of antimicrobials for food-producing species for 27 countries, between 2010 and 2016 was statistically significant with a mean decrease of 17.1%.
6. Countries that had already had a low AMC in 2010 have continued decreasing AMC by 2016, which seems to indicate that policies on AMC reduction can be extremely effective. In those countries, all those involved in the use of antimicrobials are fully aware of the need to reduce AMC.
7. In the studied period of 2010 to 2016, the reduction of overall AMC in the EU/EEA has not been translated into a statistically significant reduction of the sales of some of the HPCIA (3rd and 4th generation cephalosporins, quinolones and polymyxins). This lack of significant reduction on HPCIAs seems to suggest that policies only addressing the overall use of antimicrobials are not sufficient to result in a decrease of HPCIA and that specific measures for the decrease of those classes of antimicrobials are necessary to reduce its use.
8. The overall sales of antimicrobials in mg/kg PCU in 2016 are very strongly correlated with the sales of polymyxins in 2016 (0.861). The overall sales of antimicrobials (mg/kg PCU) in 2016 are also strongly correlated with the sales of quinolones in 2012 (0.657). The overall sales of antimicrobials in mg/kg PCU in 2016 are moderately correlated with the sales of 3rd and 4th generation cephalosporins but not within the Bonferroni cut-off limit.
9. The % of premixes are strongly correlated with the sales of polymyxins (mostly colistin), which confirms that colistin is mostly used in oral group treatments. There is a strong correlation between overall AMC and sales of polymyxins, suggesting that the reduction of the consumption of polymyxins is an important factor in reducing overall consumption of antimicrobials. Since the discovery of the *mcr-1* gene many countries have taken active policies to decrease the use of polymyxins. Following the EMA recommendations to reduce the use of colistin many EU/EEA countries have taken action to reduce such use. Although is too early to analyse the impact of those measures on use of polymyxins, the EC reports note that substantial reductions in colistin use have been achieved with few negative effects.

10. Countries with high overall sales of antimicrobials have a high use of oral forms, in particular, premixes (as total sales in mg/kg PCU, or as a % of the total sales), and vice versa, i.e. countries with small sales of antimicrobials have small sales/proportion of premixes, and oral use in general. Reducing the use of premixes (or other forms of oral group medication) in a country might be one of the most useful measures to reduce overall AMC. Increasing the percentage of use of individual treatments like injectables versus pharmaceutical forms for oral group treatment of antimicrobials could also be one of the measures to reduce overall antimicrobial use.
11. In some of the countries with high consumption of antimicrobials, this seems to be linked to the high use of antimicrobials for prophylaxis. The NVR bans in practice prophylactic use of antimicrobials for groups of animals. Eliminating unnecessary prophylactic use in groups of animals could result in a substantial reduction of AMC in animals.
12. The relationship between the sales of antimicrobials (expressed as mg/kg PCU) and the percentage of oral sales for group treatment is not linear but exponential, which seems to indicate that antimicrobials in countries with very high sales have to be administered mostly in oral group treatments as individual treatments will not result on such high consumption. The data suggest that reducing the antimicrobials administered orally for groups of animals could result in an exponential reduction of AMU.
13. Countries with a historical collection of AMC data (pre-ESVAC) have lower AMC than those that started to collect data later. But when the analysis is made according to the existence of systems collecting data by animal species, no reduction in AMC could be found, which suggests that data collection at farm level should involve most of the animal species and farms in the country and that specific AMC reduction plans by animals species should be set to reduce AMC.
14. Nearly none of the Pearson's linear correlation of the % of pigs, poultry, cattle, caprinae or the combination of pigs and poultry was statistically significant when analysed *vs* the sales of total antimicrobials in mg/kg PCU, 3rd and 4th generation cephalosporins, quinolones or polymyxins, not within the Bonferroni cut-off limit. Only the % of poultry in 2016 was moderately correlated with the sales of quinolones in 2016 within the Bonferroni cut-off limit.
15. The limited number of significant correlations between sales of antimicrobials and animal species was not expected, as more correlations with different animal production species could be expected. As noted above, this might be the result of the proxy (% of PCU of an animal species in a country) not been sufficiently detailed to allow for stronger correlations between animal species and AMC.
16. AMC is strongly linked with temperature in the EU/EEA countries, which suggests that those countries with higher environmental temperature will have higher AMC as AMR seems to increase with temperature, which (amongst other reasons) might be a factor leading to higher AMC. Countries with high environmental temperature should proactively implement policies to reduce AMC.
17. AMC data collection (and publication) is one more component of AMR plans. Data collection and publication of the results of the ESVAC project could have raised awareness of the need to reduce antimicrobial use in animals, especially with farmers and veterinarians.
18. Total sales data on AMC in animals is a powerful tool to raise awareness and knowledge on the use of antimicrobials, but it is acknowledged that data at farm level, allows for the use of better indicators, and implementation of e.g. benchmarking between farms, and better comparison of results on AMC between countries.

19. It can be concluded that multifaceted approaches like setting targets, improved biosecurity, benchmarking, vaccination and avoiding routinely use of antimicrobials seem to have strongly contributed to the reduction of antimicrobial use in animals in the EU/EEA and Switzerland, which might result on the reduction of AMR.

Annexes

12. Annexes

12.1. Annex I - Pearson linear correlations

In all the tables below year 2010 refers to the years 2010, or 2011 or 2012, as relevant. For simplicity reasons, this has been summarised as year "2010" instead of "*2010 (2011 or 2012)*".

To fit the tables, the term "cephalosporins" has been shortened as "cephas.".

Correlations that were not significant (≥ 0.05) have been left out of the table (white spaces).

The Bonferroni's significant correlations are identified by blue coloured cells.

Table 30. Pearson's linear correlations (1).

	Overall sales in mg/kg PCU 2010	Overall sales in mg/kg PCU 2016	Variation sales mg/PCU 2010 to 2016 in %	3-4 gen. Cephalos. (mg/kg PCU) 2010	3-4 gen. Cephalos.(mg/kg PCU) 2016	% Δ Cephalos.2016 to 2010	All quinolones (mg/kg PCU) 2010	All quinolones (mg/kg PCU) 2016	% Δ All quinolones 2016 to 2010	Polymyxins (mg/kg PCU) 2010	Polymyxins (mg/kg PCU) 2016	% Δ Polymyxins 2016 to 2010
Overall sales in mg/kg PCU 2010	1											
Overall sales in mg/kg PCU 2016	0.900	1										
Variation sales mg/PCU 2010-2016 in %		0.467	1									
3-4 gen. Cephalos. (mg/kg PCU) 2010				1								
3-4 gen. Cephalos.(mg/kg PCU) 2016	0.469	0.485		0.679	1							

	Overall sales in mg/kg PCU 2010	Overall sales in mg/kg PCU 2016	Variation sales mg/PCU 2010 to 2016 in %	3-4 gen. Cephalos. (mg/kg PCU) 2010	3-4 gen. Cephalos.(mg/kg PCU) 2016	% Δ Cephalos.2016 to 2010	All quinolones (mg/kg PCU) 2010	All quinolones (mg/kg PCU) 2016	% Δ All quinolones 2016 to 2010	Polymyxins (mg/kg PCU) 2010	Polymyxins (mg/kg PCU) 2016	% Δ Polymyxins 2016 to 2010
% Δ Cephalos.2016 to 2010	0.390	0.528	0.435		0.508	1						
All quinolones (mg/kg PCU) 2010	0.664	0.657					1					
All quinolones (mg/kg PCU) 2016	0.491	0.551	0.509				0.846	1				
% Δ All quinolones 2016 to 2010						0.544			1			
Polymyxins (mg/kg PCU) 2010	0.773	0.719					0.768	0.514		1		
Polymyxins (mg/kg PCU) 2016	0.819	0.861			0.384		0.792	0.693		0.872	1	
% Δ Polymyxins 2016 to 2010												1

Table 31. Pearson's linear correlations (2).

	Overall sales in mg/kg PCU 2010	Overall sales in mg/kg PCU 2016	Variation sales mg/PCU 2010 to 2016 in %	3-4 gen. Cephalos. (mg/kg PCU) 2010	3-4 gen. Cephalos.(mg/kg PCU) 2016	% Δ Cephalos.2016 to 2010	All quinolones (mg/kg PCU) 2010	All quinolones (mg/kg PCU) 2016	% Δ All quinolones 2016 to 2010	Polymyxins (mg/kg PCU) 2010	Polymyxins (mg/kg PCU) 2016	% Δ Polymyxins 2016 to 2010
Total sales (tonnes) 2016	0.526	0.578					0.615	0.457		0.836	0.773	
% Oral forms *vs* total sales 2016	0.655	0.585		0.470	0.426	0.440	0.535	0.552	0.458	0.457	0.530	
% Premixes *vs* total sales 2016	0.628	0.716	0.436				0.507	0.491		0.476	0.614	
% Oral powders *vs* total sales 2016					0.396							
% Oral solution *vs* total sales 2016								0.389				
% Injectables *vs* total sales 2016	-0.650	-0.583		-0.445	-0.415	-0.470	-0.536	-0.547	-0.459	-0.454	-0.532	
% Individual treatments *vs* total sales 2016	-0.655	-0.585		-0.470	-0.426	-0.440	-0.535	-0.552	-0.458	-0.457	-0.530	
Premix (mg/kg PCU) 2016	0.777	0.944	0.480		0.535	0.566	0.477	0.429		0.569	0.761	
Oral powder (mg/kg PCU) 2016	0.597	0.539		0.407	0.525						0.384	
Oral solution (mg/kg PCU) 2016	0.620	0.593					0.864	0.751		0.759	0.734	
Injection (mg/kg PCU) 2016	0.492	0.539			0.408	0.408				0.425	0.379	

Table 32. Pearson's linear correlations (3).

	Overall sales in mg/kg PCU 2010	Overall sales in mg/kg PCU 2016	Variation sales mg/PCU 2010-2016 in %	3-4 gen. Cephalos. (mg/kg PCU) 2010	3-4 gen. Cephalos.(mg/kg PCU) 2016	% Δ Cephalos.2016 to 2010	All quinolones (mg/kg PCU) 2010	All quinolones (mg/kg PCU) 2016	% Δ All quinolones 2016 to 2010	Polymyxins (mg/kg PCU) 2010	Polymyxins (mg/kg PCU) 2016	% Δ Polymyxins 2016 to 2010
PCU (1,000 Tonnes) 2016										0.498	0.392	
% Pigs 2016	0.386	0.372										0.463
% Poultry 2016							0.487	0.570				
% Cattle 2016		-0.386						-0.398			-0.373	
% Caprinae 2016												
% Other animals (caprinae, fish, rabbits and horses) 2016				-0.383					-0.477			
% Pigs and poultry 2016	0.481	0.439									0.428	0.521
Average Temperature (C)	0.812	0.768				0.456	0.522	0.540		0.641	0.664	
Average High Temperature (C)	0.803	0.788	0.430			0.478	0.571	0.611		0.635	0.684	
Average Low Temperature (C)	0.759	0.727				0.402	0.450	0.454		0.622	0.640	
Average Precipitation (mm)		-0.411										

Table 33. Pearson's linear correlations (4).

	Total sales (tonnes) 2016	% Oral forms *vs* total sales 2016	% Premixes *vs* total sales 2016	% Oral powders *vs* total sales 2016	% Oral solution *vs* total sales 2016	% Injectables *vs* total sales 2016	% Individual treatments *vs* total sales 2016	Premix (mg/kg PCU) 2016	Oral powder (mg/kg PCU) 2016	Oral solution (mg/kg PCU) 2016	Injection (mg/kg PCU) 2016	PCU (1,000 Tonnes) 2016
Total sales (tonnes) 2016	1											
% Oral forms *vs* total sales 2016		1										
% Premixes *vs* total sales 2016	0.373	0.485	1									
% Oral powders *vs* total sales 2016			-0.387	1								
% Oral solution *vs* total sales 2016		0.432		-0.399	1							
% Injectables *vs* total sales 2016		-0.987	-0.492		-0.406	1						
% Individual treatments *vs* total sales 2016		-1.000	-0.485		-0.432	0.987	1					
Premix (mg/kg PCU) 2016	0.476	0.416	0.781			-0.417	-0.416	1				
Oral powder (mg/kg PCU) 2016		0.445		0.675		-0.442	-0.445	0.417	1			
Oral solution (mg/kg PCU) 2016	0.680	0.561		-0.378	0.638	-0.553	-0.561	0.375		1		
Injection (mg/kg PCU) 2016		0.409					-0.409	0.511			1	

	Total sales (tonnes) 2016	% Oral forms *vs* total sales 2016	% Premixes *vs* total sales 2016	% Oral powders *vs* total sales 2016	% Oral solution *vs* total sales 2016	% Injectables *vs* total sales 2016	% Individual treatments *vs* total sales 2016	Premix (mg/kg PCU) 2016	Oral powder (mg/kg PCU) 2016	Oral solution (mg/kg PCU) 2016	Injection (mg/kg PCU) 2016	PCU (1,000 Tonnes) 2016
PCU (1,000 Tonnes) 2016	0.694									0.458		1

Table 34. Pearson's linear correlations (5).

	Total sales (tonnes) 2016	% Oral forms *vs* total sales 2016	% Premixes *vs* total sales 2016	% Oral powders *vs* total sales 2016	% Oral solution *vs* total sales 2016	% Injectables *vs* total sales 2016	% Individual treatments *vs* total sales 2016	Premix (mg/kg PCU) 2016	Oral powder (mg/kg PCU) 2016	Oral solution (mg/kg PCU) 2016	Injection (mg/kg PCU) 2016	PCU (1,000 Tonnes) 2016
% Pigs 2016		0.397				-0.367	-0.397		0.429			
% Poultry 2016		0.449			0.462	-0.447	-0.449			0.500		
% Cattle 2016			-0.377	0.435				-0.401				
% Caprinae 2016			0.464	-0.425								
% Other animals (caprinae, fish, rabbits and horses) 2016		-0.398		-0.459		0.378	0.398					
% Pigs and poultry 2016		0.538				-0.510	-0.538		0.384	0.366		
Average Temperature (C)	0.407	0.653	0.746			-0.637	-0.653	0.744	0.388	0.455	0.511	
Average High Temperature (C)	0.434	0.731	0.751			-0.714	-0.731	0.743		0.531	0.518	
Average Low Temperature (C)	0.386	0.598	0.721			-0.575	-0.598	0.718		0.397	0.518	
Average Precipitation (mm)								-0.410				

Table 35. Pearson's linear correlations (6).

	% Pigs 2016	% Poultry 2016	% Cattle 2016	% Caprinae 2016	% Other animals (caprinae, fish, rabbits and horses) 2016	% Pigs and poultry 2016	Average Temperature (C)	Average High Temperature (C)	Average Low Temperature (C)	Average Precipitation (mm)
% Pigs 2016	1									
% Poultry 2016		1								
% Cattle 2016			1							
% Caprinae 2016	-0.446		-0.514	1						
% Other animals (caprinae, fish, rabbits and horses) 2016	-0.569		-0.614	0.765	1					
% Pigs and poultry 2016	0.918	0.423		-0.425	-0.613	1				
Average Temperature (C)			-0.396	0.463			1			
Average High Temperature (C)				0.432			0.981	1		
Average Low Temperature (C)			-0.383	0.476			0.985	0.944	1	
Average Precipitation (mm)	-0.386					-0.430				1

Table 36. Bonferroni's significant correlations (<0.00147 level, 2-tailed).

Variable	Variable	Correlation	Significance (2-tailed)
% Individual treatments *vs* total sales 2016	% Oral forms *vs* total sales 2016	-1.000	< 0.00001
% Injectables *vs* total sales 2016	% Oral forms *vs* total sales 2016	-0.987	< 0.00001
% Individual treatments *vs* total sales 2016	% Injectables *vs* total sales 2016	0.987	< 0.00001
Average Low Temperature (C)	Average Temperature (C)	0.985	< 0.00001
Average High Temperature (C)	Average Temperature (C)	0.981	< 0.00001
Average Low Temperature (C)	Average High Temperature (C)	0.944	< 0.00001
Premix (mg/kg PCU) 2016	Overall sales in mg/kg PCU 2016	0.944	< 0.00001
% Pigs and poultry 2016	% Pigs 2016	0.918	< 0.00001
Overall sales in mg/kg PCU 2016	Overall sales in mg/kg PCU 2010 (2011 or 2012)	0.900	< 0.00001
Polymyxins (mg/kg PCU) 2016	Overall sales in mg/kg PCU 2016	0.861	< 0.00001
Polymyxins (mg/kg PCU) 2016	Polymyxins (mg/kg PCU) 2010 (2011 or 2012)	0.872	< 0.00001
Oral solution (mg/kg PCU) 2016	All quinolones (mg/kg PCU) 2010 (2011 or 2012)	0.864	< 0.00001
All quinolones (mg/kg PCU) 2016	All quinolones (mg/kg PCU) 2010 (2011 or 2012)	0.846	< 0.00001

Variable	Variable	Correlation	Significance (2-tailed)
Total sales (tonnes) 2016	Polymyxins (mg/kg PCU) 2010 (2011 or 2012)	0.836	< 0.00001
Average High Temperature (C)	Overall sales in mg/kg PCU 2016	0.788	< 0.00001
Average Temperature (C)	Overall sales in mg/kg PCU 2010 (2011 or 2012)	0.812	< 0.00001
Polymyxins (mg/kg PCU) 2016	Overall sales in mg/kg PCU 2010 (2011 or 2012)	0.819	< 0.00001
Average High Temperature (C)	Overall sales in mg/kg PCU 2010 (2011 or 2012)	0.803	< 0.00001
Premix (mg/kg PCU) 2016	% Premixes *vs* total sales 2016	0.781	< 0.00001
Average Temperature (C)	Overall sales in mg/kg PCU 2016	0.768	< 0.00001
% Other animals (caprinae, fish, rabbits and horses) 2016	% Caprinae 2016	0.765	< 0.00001
Total sales (tonnes) 2016	Polymyxins (mg/kg PCU) 2016	0.773	< 0.00001
Oral solution (mg/kg PCU) 2016	All quinolones (mg/kg PCU) 2016	0.751	< 0.00001
Average High Temperature (C)	% Premixes *vs* total sales 2016	0.751	< 0.00001
Average Temperature (C)	% Premixes *vs* total sales 2016	0.746	< 0.00001
Polymyxins (mg/kg PCU) 2010 (2011 or 2012)	Overall sales in mg/kg PCU 2010 (2011 or 2012)	0.773	< 0.00001

Variable	Variable	Correlation	Significance (2-tailed)
Polymyxins (mg/kg PCU) 2016	All quinolones (mg/kg PCU) 2010 (2011 or 2012)	0.792	< 0.00001
Premix (mg/kg PCU) 2016	Polymyxins (mg/kg PCU) 2016	0.761	< 0.00001
Premix (mg/kg PCU) 2016	Overall sales in mg/kg PCU 2010 (2011 or 2012)	0.777	< 0.00001
Average Temperature (C)	Premix (mg/kg PCU) 2016	0.744	< 0.00001
Average High Temperature (C)	Premix (mg/kg PCU) 2016	0.743	< 0.00001
Average Low Temperature (C)	Overall sales in mg/kg PCU 2010 (2011 or 2012)	0.759	< 0.00001
Oral solution (mg/kg PCU) 2016	Polymyxins (mg/kg PCU) 2010 (2011 or 2012)	0.759	< 0.00001
Average High Temperature (C)	% Oral forms *vs* total sales 2016	0.731	< 0.00001
Average High Temperature (C)	% Individual treatments *vs* total sales 2016	-0.731	< 0.00001
Polymyxins (mg/kg PCU) 2010 (2011 or 2012)	All quinolones (mg/kg PCU) 2010 (2011 or 2012)	0.768	< 0.00001
Average Low Temperature (C)	Overall sales in mg/kg PCU 2016	0.727	0.00001
Oral solution (mg/kg PCU) 2016	Polymyxins (mg/kg PCU) 2016	0.734	0.00001
Average Low Temperature (C)	% Premixes *vs* total sales 2016	0.721	0.00001

Variable	Variable	Correlation	Significance (2-tailed)
% Premixes *vs* total sales 2016	Overall sales in mg/kg PCU 2016	0.716	0.00001
Average High Temperature (C)	% Injectables *vs* total sales 2016	-0.714	0.00001
Average Low Temperature (C)	Premix (mg/kg PCU) 2016	0.718	0.00001
PCU (1,000 Tonnes) 2016	Total sales (tonnes) 2016	0.694	0.00002
Polymyxins (mg/kg PCU) 2010 (2011 or 2012)	Overall sales in mg/kg PCU 2016	0.719	0.00002
Polymyxins (mg/kg PCU) 2016	All quinolones (mg/kg PCU) 2016	0.693	0.00003
Oral solution (mg/kg PCU) 2016	Total sales (tonnes) 2016	0.680	0.00004
Average High Temperature (C)	Polymyxins (mg/kg PCU) 2016	0.684	0.00004
Oral powder (mg/kg PCU) 2016	% Oral powders *vs* total sales 2016	0.675	0.00008
Average Temperature (C)	Polymyxins (mg/kg PCU) 2016	0.664	0.00009
Average Temperature (C)	% Oral forms *vs* total sales 2016	0.653	0.00009
Average Temperature (C)	% Individual treatments *vs* total sales 2016	-0.653	0.00009
3-4 gen. cephalosporins (mg/kg PCU) 2016	3-4 gen. cephalosporins (mg/kg PCU) 2010 (2011 or 2012)	0.679	0.00010
Oral solution (mg/kg PCU) 2016	% Oral solution *vs* total sales 2016	0.638	0.00015
Average Temperature (C)	% Injectables *vs* total sales 2016	-0.637	0.00015

Variable	Variable	Correlation	Significance (2-tailed)
Average Low Temperature (C)	Polymyxins (mg/kg PCU) 2016	0.640	0.00019
% Oral forms *vs* total sales 2016	Overall sales in mg/kg PCU 2010 (2011 or 2012)	0.655	0.00021
% Individual treatments *vs* total sales 2016	Overall sales in mg/kg PCU 2010 (2011 or 2012)	-0.655	0.00021
All quinolones (mg/kg PCU) 2010 (2011 or 2012)	Overall sales in mg/kg PCU 2010 (2011 or 2012)	0.664	0.00022
% Injectables *vs* total sales 2016	Overall sales in mg/kg PCU 2010 (2011 or 2012)	-0.650	0.00024
All quinolones (mg/kg PCU) 2010 (2011 or 2012)	Overall sales in mg/kg PCU 2016	0.657	0.00027
% Other animals (caprinae, fish, rabbits and horses) 2016	% Cattle 2016	-0.614	0.00030
Average Temperature (C)	Polymyxins (mg/kg PCU) 2010 (2011 or 2012)	0.641	0.00031
% Pigs and poultry 2016	% Other animals (caprinae, fish, rabbits and horses) 2016	-0.613	0.00032
Average High Temperature (C)	All quinolones (mg/kg PCU) 2016	0.611	0.00033
Average High Temperature (C)	Polymyxins (mg/kg PCU) 2010 (2011 or 2012)	0.635	0.00037
% Premixes *vs* total sales 2016	Polymyxins (mg/kg PCU) 2016	0.614	0.00039

Variable	Variable	Correlation	Significance (2-tailed)
% Premixes *vs* total sales 2016	Overall sales in mg/kg PCU 2010 (2011 or 2012)	0.628	0.00045
Average Low Temperature (C)	% Oral forms *vs* total sales 2016	0.598	0.00048
Average Low Temperature (C)	% Individual treatments *vs* total sales 2016	-0.598	0.00048
Average Low Temperature (C)	Polymyxins (mg/kg PCU) 2010 (2011 or 2012)	0.622	0.00054
Oral solution (mg/kg PCU) 2016	Overall sales in mg/kg PCU 2016	0.593	0.00055
Oral solution (mg/kg PCU) 2016	Overall sales in mg/kg PCU 2010 (2011 or 2012)	0.620	0.00057
% Oral forms *vs* total sales 2016	Overall sales in mg/kg PCU 2016	0.585	0.00069
% Individual treatments *vs* total sales 2016	Overall sales in mg/kg PCU 2016	-0.585	0.00069
% Injectables *vs* total sales 2016	Overall sales in mg/kg PCU 2016	-0.583	0.00073
Total sales (tonnes) 2016	All quinolones (mg/kg PCU) 2010 (2011 or 2012)	0.615	0.00083
Total sales (tonnes) 2016	Overall sales in mg/kg PCU 2016	0.578	0.00083
Average Low Temperature (C)	% Injectables *vs* total sales 2016	-0.575	0.00088
% Poultry 2016	All quinolones (mg/kg PCU) 2016	0.570	0.00100
% Other animals (caprinae, fish, rabbits and horses) 2016	% Pigs 2016	-0.569	0.00105

Variable	Variable	Correlation	Significance (2-tailed)
Oral solution (mg/kg PCU) 2016	% Oral forms *vs* total sales 2016	0.561	0.00126
Oral solution (mg/kg PCU) 2016	% Individual treatments *vs* total sales 2016	-0.561	0.00126
Oral powder (mg/kg PCU) 2016	Overall sales in mg/kg PCU 2010 (2011 or 2012)	0.597	0.00128

12.2. Annex II - Referrals of antimicrobials

Table 37. List of main EMA/CVMP referrals on systemically (or intramammary) administered antibiotics for, amongst other, AMR reasons, including lack of efficacy (adapted from www.ema.europa.eu).

Year-end of referral	Products (or types of products) involved	Active substance	Target species	CVMP recommendation	Links to relevant web pages*
2003	Benzathine Benzylpenicillin	Benza-thine Benzylpen icillin	Cattle, sheep, pigs and horses.	The CVMP adopted an opinion confirming the recommendation for suspension of the marketing authorisations for the veterinary medicinal products.	https://ec.europa.eu/health/documents/community-register/html/vo1624.htm https://www.ema.europa.eu/en/medicines/veterinary/referrals/benzathine-benzylpenicillin-intended-administration-food-producing-species
2003	Orbax, tablets 6.25, 25 and 75 mg	Orbifloxa-cin	Dogs	The CVMP adopted a positive opinion recommending the granting of the marketing authorisation.	https://ec.europa.eu/health/documents/community-register/html/vo1627.htm https://www.ema.europa.eu/en/medicines/veterinary/referrals/orbax
2005	Micotil 300, solution for injection 300mg/ml	Tilmicosin	Cattle, sheep and rabbits	The CVMP considered that the benefit-risk profile of Micotil 300 for injection and its associated names remains positive, subject to variation of the marketing authorisations in accordance with the recommended product information.	https://ec.europa.eu/health/documents/community-register/html/vo25023.htm https://www.ema.europa.eu/en/medicines/veterinary/referrals/micotil-300-injectie

Year-end of referral	Products (or types of products) involved	Active substance	Target species	CVMP recommendation	Links to relevant web pages*
2006	Suramox 15% LA, suspension for injection 300mg/ml	Amoxici-llin	Cattle and pigs	The CVMP considered the studies submitted and concluded that withdrawal periods can be set for both cattle and pigs. The CVMP also recommended varying the marketing authorisations of the veterinary medicinal products in accordance with the Summary of Product Characteristics.	https://ec.europa.eu/health/documents/community-register/html/vo3102.htm https://www.ema.europa.eu/en/medicines/veterinary/referrals/suramox-15-la-its-associated-name-stabox-15-la
2006	Cobactan IV, powder and solvent for injection	Cefquino me sulphate	Horses	The CVMP adopted a positive opinion recommending the granting of the marketing authorisation.	https://ec.europa.eu/health/documents/community-register/html/vo2422.htm https://www.ema.europa.eu/en/medicines/veterinary/referrals/cobactan-iv-45-powder-solvent-solution-injection-its-associated-names
2006	Cobactan DC, intramammary ointment	Cefquino me sulphate	Cattle	The CVMP considered that the withdrawal period for milk should be one day after calving when the dry period is more than 5 weeks and 36 days after treatment when the dry period is 5 weeks or less and adopted a positive opinion.	https://ec.europa.eu/health/documents/community-register/html/vo2921.htm https://www.ema.europa.eu/en/medicines/veterinary/referrals/cobactan-dc-its-associated-names

Year-end of referral	Products (or types of products) involved	Active substance	Target species	CVMP recommendation	Links to relevant web pages*
2007	Doxyprex, premix	Doxycycline hyclate	Pigs	The CVMP recommended the granting of the marketing authorisation for pigs for the treatment and prevention of porcine respiratory disease, caused by *Pasteurella multocida* and *Bordetella bronchiseptica*, susceptible to doxycycline, when the disease has been diagnosed in the herd. A benefit-risk analysis could not be conducted due to the lack of pivotal evidence on clinical efficacy for the indication *M. hyopneumoniae* and recommended to remove this pathogen from the indications.	https://ec.europa.eu/health/documents/community-register/html/vo5041.htm https://www.ema.europa.eu/en/medicines/veterinary/referrals/doxyprex-100mg-premix
2007	Methoxasol T, oral solution	Trimethoprim and sulfamethoxasol	Pigs and chickens	The CVMP concluded that the benefit/risk balance of the product was positive for use in both pigs and broilers subject to recommended changes to the Summary of Product Characteristics and product information.	https://ec.europa.eu/health/documents/community-register/html/vo7581.htm https://www.ema.europa.eu/en/medicines/veterinary/referrals/methoxasol-t

Year-end of referral	Products (or types of products) involved	Active substance	Target species	CVMP recommendation	Links to relevant web pages*
2009	Enro K 10%, oral solution	Enrofloxacin	Chicken, Turkeys	The CVMP recommended that the use of the product as recommended does not constitute a risk for the environment.	https://ec.europa.eu/health/documents/community-register/html/vo11502.htm https://www.ema.europa.eu/en/medicines/veterinary/referrals/enro-k
2009	Unisol (Aviflox), oral solution	Enrofloxacin	Chicken, Turkeys	The CVMP recommended that the use of the product does not constitute a risk for the environment.	https://ec.europa.eu/health/documents/community-register/html/vo11501.htm https://www.ema.europa.eu/en/medicines/veterinary/referrals/unisol
2009	Pharmasin 100% W/W soluble granules	Tylosin tartrate	Pigs, chickens (broilers, pullets), turkeys and calves	The application did not satisfy the criteria for authorisation in respect of environmental risk. Therefore the CVMP recommended the refusal of the granting of the marketing authorisations for Pharmasin 100% W/W Water Soluble Granules and associated names.	https://ec.europa.eu/health/documents/community-register/html/vo15441.htm https://www.ema.europa.eu/en/medicines/veterinary/referrals/pharmasin

Year-end of referral	Products (or types of products) involved	Active substance	Target species	CVMP recommendation	Links to relevant web pages*
2009	Shotaflor, solution for injection	Florfeni-col	Cattle	The CVMP considered that the use of the product as recommended for therapeutic use only does not constitute a risk for the environment. However, the CVMP also considered that the wording of the therapeutic indication should be amended to clearly state the limitations of the approved use and avoid incorrect interpretation.	https://ec.europa.eu/health/documents/community-register/html/vo13722.htm https://www.ema.europa.eu/en/medicines/veterinary/referrals/shotaflor
2009	Fenflor, solution for injection	Florfeni-col	Cattle	The CVMP considered that the use of the product as recommended for therapeutic use only does not constitute a risk for the environment. However, the CVMP also considered that the wording of the therapeutic indication as authorised in the Reference Member State should be amended to clearly state the limitations of the approved use and avoid incorrect interpretation.	https://ec.europa.eu/health/documents/community-register/html/vo13721.htm https://www.ema.europa.eu/en/medicines/veterinary/referrals/fenflor

Year-end of referral	Products (or types of products) involved	Active substance	Target species	CVMP recommendation	Links to relevant web pages*
2010	Colistin, 2MIU/ml concentrate for oral solution	Colistin	All food-producing species	The CVMP recommended variations of the marketing authorisations for veterinary medicinal formulations containing colistin at 2 000 000 IU per ml and intended for administration in drinking water to food-producing species in order to amend the SPC, labelling and package leaflet to harmonise the posology and withdrawal periods	https://ec.europa.eu/health/documents/community-register/html/vo16002.htm https://www.ema.europa.eu/en/medicines/veterinary/referrals/colistin
2010	Quinolones/ fluoroquinolones	Quinolo-nes/ Fluoroqui nolones	All food-producing species	The CVMP recommended variations to the terms of the marketing authorisations for veterinary medicinal products containing (fluoro)quinolones intended for food-producing species where it has been identified that the SPC and package leaflet have not been updated in line with the precautionary phrases in the CVMP "Reflection paper on the use of fluoroquinolones in food-producing animals – Precautions for use in the SPC regarding prudent use guidance" (EMEA/CVMP/416168/2006) [242]	https://ec.europa.eu/health/documents/community-register/html/vo16181.htm https://www.ema.europa.eu/en/medicines/veterinary/referrals/quinolones

Year-end of referral	Products (or types of products) involved	Active substance	Target species	CVMP recommendation	Links to relevant web pages*
2011	Doxycycline 50% WSP and associated names	Doxycycline hyclate	Poultry, cattle and pigs	The CVMP considered that the benefit/risk profile for Doxycycline 50% WSP and associated names remains positive subject to variation of the marketing authorisations in accordance with the summary of product characteristics.	https://ec.europa.eu/health/documents/community-register/html/vo20221.htm https://www.ema.europa.eu/en/medicines/veterinary/referrals/doxycycline-50-wsp
2012	Baytril 10% oral solution and associated names	Enrofloxacin	Poultry, rabbits	The CVMP considered that the benefit-risk profile of Baytril 10% oral solution and its associated names remain positive, subject to variation of the marketing authorisations in accordance with the recommended product information, and to changing a condition on the marketing authorisations.	https://ec.europa.eu/health/documents/community-register/html/vo24243.htm https://www.ema.europa.eu/en/medicines/veterinary/referrals/baytril-10-oral-solution

Year-end of referral	Products (or types of products) involved	Active substance	Target species	CVMP recommendation	Links to relevant web pages*
2012	VMPs containing systemically administered (parenteral or oral) 3rd and 4th generation cephalosporins intended for use in food-producing species	Ceftiofur, cefquino-me and cefoperazone	All food-producing species	The CVMP considered that the overall benefit-risk balance for these products remains positive subject to the recommended changes of the product information and that variations are necessary to the terms of the marketing authorisation for all veterinary medicinal products containing systemically administered (parenteral and oral) 3rd and 4th generation cephalosporins intended for use in food-producing species.	https://ec.europa.eu/health/documents/community-register/html/vo22101.htm https://www.ema.europa.eu/en/medicines/veterinary/referrals/cephalosporins
2012	Hipralona Enro-S and its generics	Enrofloxacin	Rabbits	The CVMP recommended that the marketing authorisations for the veterinary medicinal product Hipralona Enro-S and its generics should be maintained.	https://ec.europa.eu/health/documents/community-register/html/vo23802.htm https://www.ema.europa.eu/en/medicines/veterinary/referrals/hipralona-enro-s
2013	Nuflor Swine Once 450 mg/ml	Florfeni-col	Pigs	The CVMP recommended the refusal of the granting of the marketing authorisations and the suspension of the existing marketing authorisations.	https://ec.europa.eu/health/documents/community-register/html/vo24893.htm https://www.ema.europa.eu/en/medicines/veterinary/referrals/nuflor-swine-once

Year-end of referral	Products (or types of products) involved	Active substance	Target species	CVMP recommendation	Links to relevant web pages*
2014	Suanovil 20 and associated names, Captalin and associated names and generic products thereof, including pending applications	Spiramy-cin	Cattle, calves and pigs	The CVMP considered that the overall benefit-risk profile for these products remains positive subject to amendments in the product information.	https://ec.europa.eu/health/documents/community-register/html/vo25353.htm https://www.ema.europa.eu/en/medicines/veterinary/referrals/suanovil-20-captalin-associated-names-generic-products-thereof
2014	Linco-Spectin 100 and its associated names	Lincomy-cin, spectinom ycin	Pigs, chickens	The CVMP considered that the benefit-risk profile of Linco-Spectin 100 and its associated names remains positive, subject to variation of the marketing authorisations in accordance with the recommended product information.	https://ec.europa.eu/health/documents/community-register/html/vo25233.htm https://www.ema.europa.eu/en/medicines/veterinary/referrals/linco-spectin-100-associated-names
2014	All veterinary medicinal products containing enrofloxacin to be administered via the drinking water to chickens and turkeys	Enrofloxa cin	Chickens, turkeys	The Agency's Committee for Medicinal Products for Veterinary Use (CVMP) concluded that these products should no longer be used in chickens and turkeys to treat *Escherichia coli* infections and that the product information for the products should be amended accordingly.	https://ec.europa.eu/health/documents/community-register/html/vo25077.htm https://www.ema.europa.eu/en/medicines/veterinary/referrals/veterinary-medicines-containing-enrofloxacin-be-administered-drinking-water-chickens-andor-turkeys

Year-end of referral	Products (or types of products) involved	Active substance	Target species	CVMP recommendation	Links to relevant web pages*
2014	Baytril 2.5% injectable, Baytril 5% injectable, Baytril 10% injectable and associated names, and related veterinary medicinal products authorised under Article 13 of Directive 2001/82/EC	Enrofloxacin	Food-producing species and companion animals	The CVMP considered that the benefit-risk profile of Baytril 10% oral solution and its associated names remains positive, subject to variation of the marketing authorisations in accordance with the recommended product information, and subject to a condition on the marketing authorisations.	https://ec.europa.eu/health/documents/community-register/html/vo24243.htm https://www.ema.europa.eu/en/medicines/veterinary/referrals/baytril-10-oral-solution
2014	All veterinary medicinal products containing tylosin to be administered orally via feed or the drinking water to pigs	Tylosin	Pigs	The CVMP considered that the overall benefit-risk profile for these products remains positive subject to amendments in the product information	https://ec.europa.eu/health/documents/community-register/html/vo25251.htm https://www.ema.europa.eu/en/medicines/veterinary/referrals/veterinary-medicinal-products-containing-tylosin-be-administered-orally-feed-drinking-water-pigs

Year-end of referral	Products (or types of products) involved	Active substance	Target species	CVMP recommendation	Links to relevant web pages*
2014	Resflor solution injectable	Florfenicol, flunixin	Cattle	The CVMP concluded that the clinical benefit of Resflor in the treatment of respiratory infections associated with *M. bovis* has been demonstrated and no specific risk of AMR has been identified with the use of this product.	https://ec.europa.eu/health/documents/community-register/html/vo25387.htm https://www.ema.europa.eu/en/medicines/veterinary/referrals/resflor-solution-injectable-associated-names
2015	All veterinary medicinal products containing gentamicin presented as solutions for injection to be administered to horses	Gentamicin	Horses	The CVMP recommended variations to the terms of the marketing authorisations for veterinary medicinal products containing gentamicin presented as solutions for injection to be administered to horses.	https://ec.europa.eu/health/documents/community-register/html/vo25429.htm https://www.ema.europa.eu/en/medicines/veterinary/referrals/gentamicin
2015	All veterinary medicinal products containing colistin to be administered orally	Colistin	All food-producing species	The CVMP considered that the overall benefit-risk profile for these products remains positive subject to amendments in the product information.	https://ec.europa.eu/health/documents/community-register/html/vo25478.htm https://www.ema.europa.eu/en/medicines/veterinary/referrals/colistin-oral

Year-end of referral	Products (or types of products) involved	Active substance	Target species	CVMP recommendation	Links to relevant web pages*
2016	All veterinary medicinal products containing a combination of lincomycin and spectinomycin to be administered orally to pigs and/or poultry	Lincomy-cin and spectinom ycin	Pigs and poultry	The CVMP concluded that the overall benefit-risk balance for premixes for medicated feeding stuff and powders to be administered with the feed containing a combination of lincomycin and spectinomycin is negative, as the use of these products at the recommended dosing regimens entails a high risk of resistance selection and development due to exposure to low antimicrobial levels for prolonged periods. The CVMP recommended that all marketing authorisations for premixes for medicated feeding stuff and powders to be administered with the feed containing a combination of lincomycin and spectinomycin be withdrawn throughout the EU.	https://ec.europa.eu/health/documents/community-register/html/vo25971.htm https://www.ema.europa.eu/en/medicines/veterinary/referrals/veterinary-medicinal-products-containing-combination-lincomycin-spectinomycin-be-administered-orally

Year-end of referral	Products (or types of products) involved	Active substance	Target species	CVMP recommendation	Links to relevant web pages*
2016	All veterinary medicinal products containing colistin in combination with other antimicrobial substances to be administered orally	Colistin in combination with other antimicro-bial substances	All food-producing species	The CVMP concluded that the overall benefit-risk balance for the aforementioned products is negative, due to a lack of clinical relevance and in view of over-exposure of colistin that could pose a potential risk to animal and human health from an acceleration of the occurrence of colistin resistance. The CVMP recommended that all marketing authorisations for veterinary medicinal products containing colistin in combination with other antimicrobial substances to be administered orally should be withdrawn throughout the EU.	https://ec.europa.eu/health/documents/community-register/html/vo25976.htm https://www.ema.europa.eu/en/medicines/veterinary/referrals/colistin-combinations
2017	Denagard 45% and associated names	Tiamulin fumarate	Pigs, chickens, turkeys	The CVMP concluded that there is a need to harmonise the product information SPC, labelling and package leaflet) for Denagard 45% in the EU.	https://ec.europa.eu/health/documents/community-register/html/vo26282.htm https://www.ema.europa.eu/en/medicines/veterinary/referrals/denagard-45

Year-end of referral	Products (or types of products) involved	Active substance	Target species	CVMP recommendation	Links to relevant web pages*
2017	All veterinary medicinal products containing gentamicin as solution for injection for cattle and pigs	Gentami-cin	Cattle, pigs	The EMA completed a review of the consumer safety of the withdrawal periods for cattle (meat and milk) and pigs (meat and offal) for veterinary medicinal products containing gentamicin presented as solutions for injection. The Agency's CVMP concluded that the overall benefit-risk balance for veterinary medicinal products containing gentamicin presented as solutions for injection is positive and recommended amendments to withdrawal periods for cattle and pigs to provide assurance for consumer safety.	https://ec.europa.eu/health/documents/community-register/html/vo26159.htm https://www.ema.europa.eu/en/medicines/veterinary/referrals/veterinary-medicinal-products-containing-gentamicin-presented-solutions-injection-be-administered

Year-end of referral	Products (or types of products) involved	Active substance	Target species	CVMP recommendation	Links to relevant web pages*
2017	VMPs containing tylosin to be administered parenterally and intended for the treatment of bovine mastitis caused by *Mycoplasma* spp	Tylosin	Cattle	The CVMP concluded that, in the absence of pre-clinical or clinical data, treatment of bovine mastitis caused by *Mycoplasma* spp. with the aforementioned veterinary medicinal products is not effective. The CVMP recommended deletion of the indications related to 'bovine mastitis caused by *Mycoplasma* spp.' or 'bovine mastitis caused by *Mycoplasma bovis*' from the product information for the products concerned.	https://ec.europa.eu/health/documents/community-register/html/vo26277.htm https://www.ema.europa.eu/en/medicines/veterinary/referrals/tylosin-injection-mastitis
2017	Lincocin and associated names	Lincomy-cin	Pigs and chickens	The CVMP concluded that there is a need to harmonise the product information (SPC, labelling and package leaflet) for Lincocin in the EU	https://ec.europa.eu/health/documents/community-register/html/vo26357.htm https://www.ema.europa.eu/en/medicines/veterinary/referrals/lincocin-its-associated-names
2018	Girolan and its associated name Apralan	Apramy-cin sulfate	Calves, pigs, chickens and rabbits	The CVMP concluded that there is a need to harmonise the product information (SPC, labelling and package leaflet) for the aforementioned product in the EU	https://ec.europa.eu/health/documents/community-register/html/vo26433.htm https://www.ema.europa.eu/en/medicines/veterinary/referrals/girolan-its-associated-name-apralan

* First web link refers to the EU Union Register of medicinal products web page, the second web link to the EMA web page on the referral.

12.3. Annex III - EC prudent use recommendations

Table 38. EC prudent use recommendations and considerations on EU/MS actions taken.

EC prudent use recommendations	Considerations on EU/MSs actions taken
The prescription and dispensation of antimicrobials must be justified by a veterinary diagnosis in accordance with the current status of scientific knowledge.	From the on-farm information on reasons for the use of antibiotics it seems evident that prophylactic use is relatively common in some MSs, so although the veterinary prescription might exist might not be according to responsible use principles.
Where it is necessary to prescribe an antimicrobial, the prescription should be based on a diagnosis made following clinical examination of the animal by the prescribing veterinarian. Where possible, antimicrobial susceptibility testing should be carried out to determine the choice of antimicrobial.	The need for the veterinarian to diagnose the animal disease following the clinical examination of the animal is recommended in prudent use guidelines [32, 46, 138], it is acknowledged that in some cases and due to the need to act rapidly an AST might not be possible.
Antimicrobial metaphylaxis should be prescribed only when there is a real need for treatment. In such cases, the veterinarian should justify and document the treatment on the basis of clinical findings on the development of a disease in a herd or flock. Antimicrobial metaphylaxis should never be used in place of good management practices.	The metaphylactic use of antimicrobials is one of the recurrent subjects of all the recommendations on the responsible use of antimicrobials. Metaphylaxis (and prophylaxis) use of antimicrobials is one of the factors that have the most significant impact on the use of antimicrobials in animals.
Routine prophylaxis must be avoided. Prophylaxis should be reserved for exceptional case-specific indications.	As indicated above, the prophylactic use of antimicrobials is one of the main reasons for the high use of antimicrobials. The new veterinary medicines legislation and the legislation on medicated feedstuff recommends banning most of the prophylactic use of antimicrobials. However, a blanket ban of prophylactic use of antimicrobials could ban certain prophylactic use of antimicrobials in animals that are legitimate like the targeted dry cow therapy or the perioperative use of antimicrobials in immunodepressed animals, so there good reasons to allow the use of antimicrobials for prophylactic reasons in certain cases.

EC prudent use recommendations	Considerations on EU/MSs actions taken
Administering medication to an entire herd or flock should be avoided whenever possible. Sick animals should be isolated and treated individually (e.g. by administrating injectables).	The way in which antimicrobials are administered varies significantly between countries (see 9.3.1. on different use of pharmaceutical forms of antimicrobials per country). The group administration form of antimicrobials (e.g. premixes or soluble or oral powders) are the forms most used in those countries with a high AMC per biomass.
All information relating to the animals, the cause and the nature of the infection and the range of available antimicrobial products must be taken into account when making a decision regarding antimicrobial treatment.	Further strengthening of the one to one relationship between the animal owner and the veterinarian seems to be one of the reasons that favour decreasing the antimicrobial use [125, 130]. More than 95 percent of pigs in Denmark are covered by Veterinary Advisory Service Contracts between farmers and veterinarians [206]
A narrow-spectrum antimicrobial should always be the first choice unless prior susceptibility testing — where appropriate supported by relevant epidemiological data — shows that this would be ineffective.	Implementation of this recommendation will depend on the availability of narrow-spectrum antimicrobials authorised in the country, due to factors like size market or economic interests, some of those formulations might not be available for use by the veterinarians.
The use of broad-spectrum antimicrobials and antimicrobial combinations should be avoided (with the exception of fixed combinations contained in authorised veterinary medicinal products).	The role of combinations of antimicrobials is disputable, with many of them been combinations of CIAs. The CVMP has recommended banning the use of combinations of colistin with other substances as well as other combinations [154].
If an animal or group of animals suffer from recurrent infection(s) requiring antimicrobial treatment, efforts should be made to eradicate the strains of the micro-organisms by determining why the disease is recurring and altering the production conditions, animal husbandry and/or management.	Some MSs have obliged farmers to set contracts with veterinarians in order to improve the knowledge of the epidemiological situation on the field and favour a better use of antimicrobials [125, 130].
Use of antimicrobial agents prone to propagate transmissible resistance should be minimised.	Some MSs have banned or restricted, the use of certain HPCIAs [125, 126, 131, 134]. The EMA has also produced recommendations on the need to reduce the use of colistin [113-116].

EC prudent use recommendations	Considerations on EU/MSs actions taken
A number of compounds on the World Health Organisation's list of CIAs are only authorised in medicinal products for human use. As laid down in EU legislation, those that do not have marketing authorisations as veterinary medicinal products for use in food-producing animals may only be used off-label (following the cascade) in these animals if the substance in question is listed in Table 1 of the Annex to Commission Regulation (EU) No 37/2010	The New Veterinary Regulation, Regulation (EU) 2019/6 addresses in detail such requirement [1, 260]
The off-label use (cascade) of the compounds referred to above for non-food-producing animals (e.g. pets and animals used for sports) should be avoided and strictly limited to very exceptional cases, e.g. where there are ethical reasons for doing so, and only when laboratory AST have confirmed that no other antimicrobial would be effective.	The use of antimicrobials in food-producing species and companion animals has different motivations, whilst animal welfare should be a common subject. In food-producing animals, the economic benefits are the driving force, whilst for companion animals, the welfare of the animal is the driving force. Traditionally the problem of AMR has been focused on food-producing species (and the use of antimicrobials in humans), whilst other sources of antimicrobials, e.g. used for companion animals, in agriculture or in the production of biofuels has received limited attention; AMR from production of biofuel are potentially important issues that have been neglected or somewhat marginalised in debates around biofuels and may need to be considered [102, 103]. The CVMP has published a paper on off label use of antimicrobials [261] with recommendations about such use.
The need for antimicrobial therapy should be reassessed on a regular basis to avoid unnecessary medication.	The systematic, not needed use of antimicrobials is one of the more significant concerns of the use of antimicrobials in animals. Benchmarking seems to be one of the most appropriate measures to tackle such inappropriate use [89]. Some countries have reported unnecessary systematic use of antimicrobials [123, 124, 144].
The perioperative use of antimicrobials should be minimised by using aseptic techniques.	The practice of perioperative use of antimicrobials is subject to debate, and there are different practices in different EU MSs [139, 262-264]

EC prudent use recommendations	Considerations on EU/MSs actions taken
When possible, alternative strategies for controlling diseases that have been proven to be equally efficient and safe (e.g. vaccines) should be preferred over antimicrobial treatment.	Alternatives to the use of antimicrobials should be promoted in order to decrease antimicrobial use. See the CVMP strategy on antimicrobials [14, 47, 48], RONAFA [139] and the JIACRA opinions [6, 7] for detailed recommendations. Postma *et al.,* 2015 [265] in a study involving 111 pig health experts from Belgium, Denmark, France, Germany, Sweden and Switzerland found that the top 5 measures in terms of perceived effectiveness were (1) improved internal biosecurity, (2) improved external biosecurity, (3) improved climate/environmental conditions, (4) high health/Specific Pathogen Free/disease eradication and (5) increased vaccination
The pharmacovigilance system should be used to obtain information and feedback on therapeutic failures, so as to identify potential resistance issues in the case of use of existing, new or alternative treatment options.	Unfortunately, it seems that few data are obtained through the pharmacovigilance systems on lack of efficacy. More data will be required to understand how pharmacovigilance can be used to fight against AMR [266]
A network of laboratories with the capacity for performing ASTs in zoonotic and commensal micro-organisms and target pathogens should be established in each Member State to ensure the availability of susceptibility testing.	EC legislation has resulted in improved surveillance of antimicrobial zoonotic and commensal micro-organisms. However, there is a lack of official networks on target pathogens.
The instruction given in the product information (SPC, leaflet, labelling) and by the veterinarian must be complied with, both in terms of dosage and duration of treatment.	Some studies have demonstrated that the use of antimicrobials differs in many cases from the SPC recommendations. Updating the SPC of antimicrobials is a pending activity (see CVMP strategy on antimicrobials) [47, 48].

12.4. Annex IV – Summary of the AMEG categorisation

Table 39. Adapted summary of the AMEG categorisation [9].

AMEG Categories	Antimicrobial class, subclasses, substances
Category A (*"Avoid"*)	Amdinopenicillins Carbapenems and other penems Cephalosporins, Other cephalosporins and penems (ATC code J01DI) Glycopeptides Glycylcyclines Lipopeptides Monobactams Oxazolidinones Penicillins: carboxypenicillins and ureidopenicillins combinations with β-lactamase inhibitors Phosphonic acid derivates (e.g. fosfomycin) Pseudomonic acid Riminofenazines Streptogramins Sulfones Drugs used solely to treat tuberculosis or other mycobacterial diseases
Category B (*"Restrict"*)	Cephalosporins, 3rd- and 4th-generation Polymyxins (e.g. colistin) Quinolones (fluoroquinolones and other quinolones)
Category C (*"Caution"*)	Aminoglycosides and aminocyclitol Aminopenicillins in combination with β-lactamase inhibitors (e.g. amoxicillin-clavulanic acid) Amphenicols (florfenicol & thiamphenicol) Cephalosporins, 1st- and 2nd-generation and cephamycins Macrolides Lincosamides Pleuromutilins Rifamycins

Category D (*"Prudence"*)	Aminopenicillins, without β-lactamase inhibitors Cyclic polypeptides (bacitracin) Nitrofuran derivatives (e.g. nitrofurantoin)* Nitroimidazoles* Penicillins: Anti-staphylococcal penicillins (β-lactamase-resistant penicillins) Penicillins: Natural, narrow-spectrum penicillins (β-lactamase-sensitive penicillins) Steroid antibacterials (fusidic acid)* Sulfonamides, dihydrofolate reductase inhibitors and combinations Tetracyclines (* Authorised for companion animals only)

12.5. Annex V – Scope of ESVAC data

Table 40. Variables reported to ESVAC for each antimicrobial veterinary medicinal product.

	Variable	Description of variable	Justification
	Country	ISO code (http://www.iso.org/iso/country_codes)	To identify the place of collected sales data.
	Year		To identify the time period for the collected sales data.
Product information	MA	Marketing authorisation number	To allow for the unique identification of the veterinary medicinal product (VMP) and enable a link with other databases. To allow for market analysis if all the products are available.
	ID	Medicinal product package code value Digit code is a unique identifier for each package size, strength and formulation of the VMP. Because it is a key variable in many databases, it must be stable over time, i.e. so that VMPs no longer available on the market or that are no longer registered can still be identified to allow for analysis of historical data.	To allow for analysis of historical data. To allow for the identification of duplicate reporting of sales.
	Name	Medicinal product name (in the national language) e.g. Harmony vet tablets 2 × 30; Harmony vet long-acting injection 10 ml.	For validation purposes.
	Form	Pharmaceutical form Bolus (BOLUS), Injection (INJ), Intramammary preparation (INTRAMAM), Intramammary preparation dry cow (INTRAMAM-DC), Oral solution (ORAL SOLU), Oral paste (ORAL PASTE), Oral powder (ORAL POWD), Premix (PREMIX),	Important to avoid misinterpretation of the pharmaceutical form if given in a language other than English. Allows for reporting of data as individual or group treatment.

	Variable	Description of variable	Justification
		Capsules and Tablets, etc. (TABL), Intrauterine preparation (INTRAUT).	
	Long-acting	Long-acting injectable preparations This refers to injectable preparations that - once injected, maintain their antimicrobial activity over a long period of time.	Optional.
	Packsize	Content quantity in package: pack size (numerical only) e.g. 100 for 100 tablets or 100 intramammary prep.; 10 for 10 ml injection; Package of 2 kg premix: 2; Box of 10 blisters of 30 tablets: 300; Box of 12 injectors: 12.	To allow for calculation of the amount of active ingredient in each package/product.
	PacksizeU	Content unit of measurement E.g. ML, L, G, KG, PIECE (for example, for tablets, capsules, boluses and intramammary prep.).	To allow for calculation of the amount of active ingredient in each package/product.
	ATCvet - 5th level	ATCvet: Anatomical Therapeutic Chemical (Classification) Veterinary WHO ATCvet code last version to be used.	Generally, a classification system needs to have a common language when reporting use and analysing data with data on AMR, e.g. for 3rd- and 4th-generation cephalosporins. To have a common language for defining confidentiality of the data (can be converted into ATCvet 3rd level).
	Species	Animal species All the animal species for which the VMP is approved, e.g. cattle (CA), poultry (POU).	Optional.

	Variable	Description of variable	Justification
	No sold	Number of packages sold/year/country	To calculate the weight of the active ingredient sold.
Ingredient	Ingr	Active ingredient name (ATCvet name) In the case of multi-ingredient VMP, the ATCvet names of all the ingredients must be given.	Important to avoid misinterpretation of ingredient name if given in a language other than English. Use of ATCvet names facilitates the identification of active ingredients as well as standardised reporting.
	Salt	Salt of the active ingredient E.g. colistin sulfate and colistin methanesulfonate.	Only in cases when the strength is given in IU, IU/ML or IU/UNIT and when different salts exist, to allow for conversion to the weight of the active ingredient.
	Prodrug	Prodrug name (ATCvet name) E.g. procaine penicillin which is the prodrug for benzylpenicillin.	Only in cases when a product contains a prodrug.
	Strength	Quantity of the active ingredient in each unit as declared in SPC/label: strength (numerical only) e.g. 10 for 10 MG/TABLET, 10 IU/TABLET, 10 MG/ML, 10 IU/ML, 10 MG/PIECE or 10 IU/PIECE. In case of a multi-ingredient VMP, strength must be given for each ingredient separately.	To allow for calculation of the amount of active ingredient in each package/product and to validate INGR CONTENT.
	StrengthU	Unit of measurement for strength E.g. IU, IU/G, IU/ML, IU/PIECE, G, G/KG, G/L, MG, MG/ML, MG/PIECE. In case of a multi-ingredient VMP, unit of measurement strength has to be given for each ingredient on a separate line.	To allow for calculation of the amount of active ingredient in each package/product and to validate INGR CONTENT.

	Variable	Description of variable	Justification
	Conv Fact IU	Conversion factor IU When strength is given as IU, IU/ML or IU/PIECE.	When strength is only given as IU, IU/ML or IU/PIECE. To allow for the calculation of the weight of the active ingredient in the package.
	Conv Fact Prodr	Conversion factor prodrug Only when strength is given for the prodrug and not for the active ingredient (e.g. procaine penicillin that is prodrug for benzylpenicillin).	To allow for calculation of the weight of the active ingredient in the package.
	Ingr content	Content of active ingredient in the package In case of a multi-ingredient VMP, the content in the package has to be given separately for each ingredient on a separate line.	Optional: To allow for validation of the ESVAC calculations.
	Cont unit (G)	Unit of the active ingredient in the package To be given in grams (g) for all substances. In case of a multi-ingredient VMP, the content unit has to be given separately for each ingredient on a separate line.	Optional: to allow for validation of the ESVAC calculations.
	Tonnes sold	Tonnes sold of the active ingredient	

Table 41. Categories and ATCvet codes of antimicrobial veterinary medicinal products included in the data.

Categories of veterinary antimicrobial agents	ATCvet codes
Antimicrobial agents for intestinal use	QA07AA; QA07AB
Antimicrobial agents for intrauterine use	QG01AA; QG01AE; QG01BA; QG01BE; QG51AA; QG51AG
Antimicrobial agents for systemic use	QJ01
Antimicrobial agents for intramammary use	QJ51

Categories of veterinary antimicrobial agents	ATCvet codes
Antimicrobial agents for antiparasitic use (solely sulphonamides	QP51AG

12.6. Annex VI – Weights used to calculate the population correction unit

Table 42. Weights used to calculate the population correction unit are collected from guidelines on environmental risk assessment.

Animal category	Weight in kg
Slaughtered or livestock (Eurostat)	
Slaughtered cow	425
Slaughtered heifer	200
Slaughtered bullocks and bulls	425
Slaughtered calves and young cattle	140
Dairy cow	425
Slaughtered pig	65
Living sow	240
Broiler	1
Turkey	6.5
Slaughtered sheep and goats	20
Living sheep	75
Horse	400
Rabbit	1.4
Imported/exported for fattening or slaughter (TRACES data)	
Slaughtered bovine	425
Fattening bovine	140
Slaughtered pig	65
Fattening pig	25
Slaughtered poultry	1
Slaughtered sheep	20
Fattening sheep	20
Slaughtered goat	20
Fattening goat	20

Glossary

13. Glossary

AGP:	Antimicrobial Growth Promoter
AMC:	Antimicrobial Consumption
AMEG:	Antimicrobial Advice Ad Hoc Expert Group
AMR:	Antimicrobial Resistance
ANMV:	French Agency for Veterinary Medicinal Products
ANSES:	French Agency for Food, Environmental and Occupational Health & Safety
AST:	Antimicrobial Susceptibility Testing
ATCvet:	Anatomical Therapeutic Chemical animals, classification system for veterinary medicinal products
CHMP:	Committee for Medicinal Products for Human Use
CIA:	Critically Important Antimicrobial
CVMP:	Committee of Medicinal Products for Veterinary Use
DART:	German Strategy against Antimicrobial Resistance
DDDs:	Defined Daily Doses
DCDvet:	assumed average dose per kg animal per species per treatment course
DDDvet:	assumed average dose per kg animal per species per day
DF:	Degree of Freedom
EC:	European Commission
ECDC:	European Centre for Disease Prevention and Control
EEA:	European Economic Area
EFSA:	European Food Safety Authority
EMA:	European Medicines Agency
ESBL:	Extended-spectrum β-lactamases
ESCMID:	European Society of Clinical Microbiology and Infectious Diseases
ESVAC:	European Surveillance of Veterinary Antimicrobial Consumption
EU:	European Union
FAO:	Food and Agriculture Organization
FDA:	Food and Drug Administration
FVE:	Federations of Veterinarians of Europe
G7:	Group of Seven
HPCIA:	Highest Priority Critically Important Antimicrobials
JIACRA:	Joint Interagency Antimicrobial Consumption and Resistance Analysis)
MA:	Marketing Authorisation

MCR-1:	Plasmid-mediated Colistin Resistance Mechanism
MDR:	Multidrug-Resistant
MRL:	Maximum Residue Limit
MRSA:	Methicillin-resistant *Staphylococcus aureus*
MRSP:	Methicillin-resistant *Staphylococcus pseudintermedius*
MS:	Member State (a country that belongs to the European Union).
OECD:	Organisation for Economic Co-operation and Development
OIE:	World Organisation for Animal Health
PCU:	Population Correction Unit
PK/PD:	Pharmacokinetics/Pharmacodynamics.
RONAFA:	(Opinion) to reduce the need to use antimicrobial agents in animal husbandry in the European Union, and the resulting impacts on food safety
SCENIHR	Scientific Committee on Emerging and Newly Identified Health Risks
SPC:	Summary of Product Characteristics.
SPSS:	IBM SPSS Statistics for Windows/Macintosh, Version 25.0. Armonk, NY: IBM Corp. (formerly Statistical Package for the Social Sciences)
STD:	Standard Deviation.
TATFAR:	Trans-Atlantic Task Force on Antimicrobial Resistance.
TB:	Tuberculosis
TFAMR:	Ad hoc Codex Intergovernmental Task Force on Antimicrobial Resistance
TRACES:	TRAde Control and Expert System
UAB:	Universitat Autònoma de Barcelona/University Autonomous of Barcelona
UN:	United Nations.
VCIA:	Veterinary Critically Important Antimicrobials
VHIA:	Veterinary Highly Important Antimicrobials
VIA:	Veterinary Important Antimicrobials
VMP:	Veterinary Medicinal Product
WHO:	World Health Organisation

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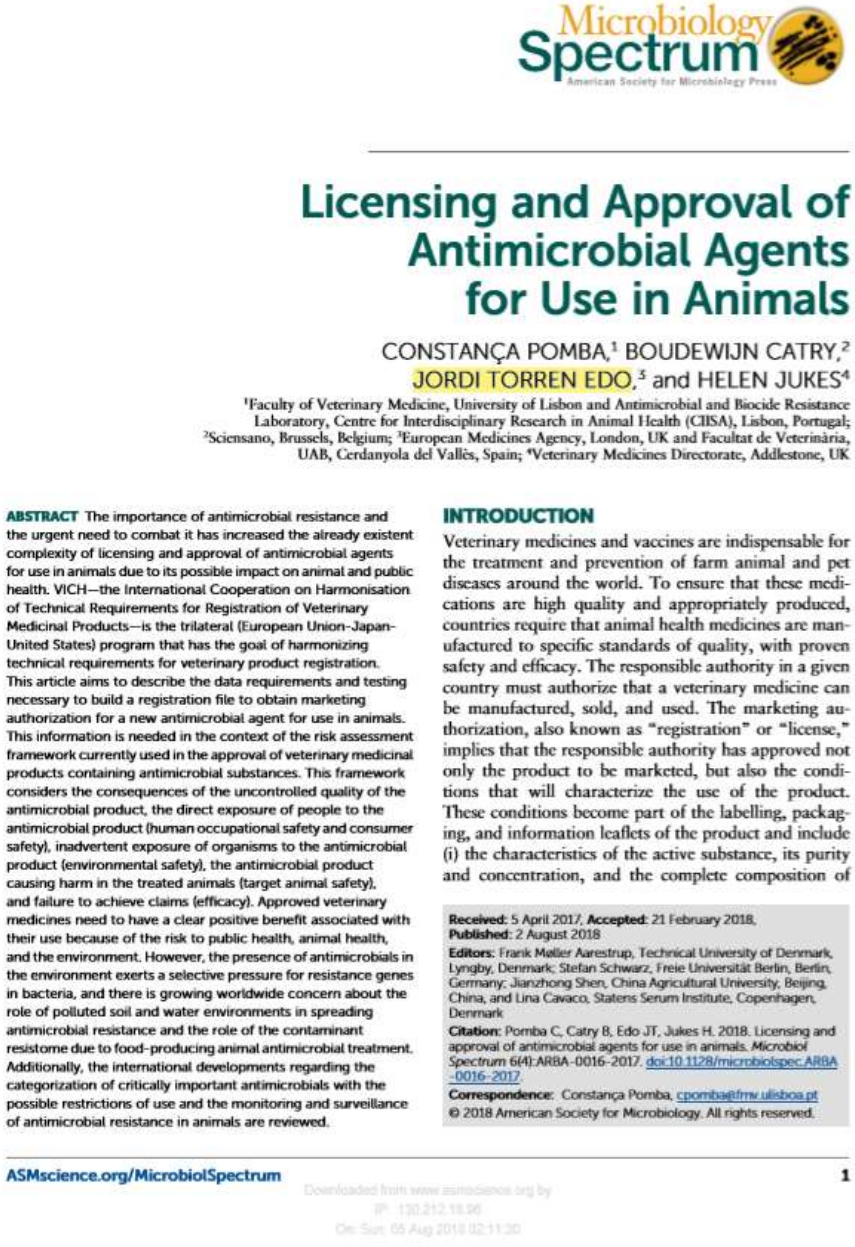
Publications

17. Publications

The first page[3] of relevant publications co-authored are attached for informative purposes only.



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Licensing and Approval of Antimicrobial Agents for Use in Animals

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ABSTRACT The importance of antimicrobial resistance and the urgent need to combat it has increased the already existent complexity of licensing and approval of antimicrobial agents for use in animals due to its possible impact on animal and public health. VICH—the International Cooperation on Harmonisation of Technical Requirements for Registration of Veterinary Medicinal Products—is the trilateral (European Union-Japan-United States) program that has the goal of harmonizing technical requirements for veterinary product registration. This article aims to describe the data requirements and testing necessary to build a registration file to obtain marketing authorization for a new antimicrobial agent for use in animals. This information is needed in the context of the risk assessment framework currently used in the approval of veterinary medicinal products containing antimicrobial substances. This framework considers the consequences of the uncontrolled quality of the antimicrobial product, the direct exposure of people to the antimicrobial product (human occupational safety and consumer safety), inadvertent exposure of organisms to the antimicrobial product (environmental safety), the antimicrobial product causing harm in the treated animals (target animal safety), and failure to achieve claims (efficacy). Approved veterinary medicines need to have a clear positive benefit associated with their use because of the risk to public health, animal health, and the environment. However, the presence of antimicrobials in the environment exerts a selective pressure for resistance genes in bacteria, and there is growing worldwide concern about the role of polluted soil and water environments in spreading antimicrobial resistance and the role of the contaminant resistome due to food-producing animal antimicrobial treatment. Additionally, the international developments regarding the categorization of critically important antimicrobials with the possible restrictions of use and the monitoring and surveillance of antimicrobial resistance in animals are reviewed.

INTRODUCTION

Veterinary medicines and vaccines are indispensable for the treatment and prevention of farm animal and pet diseases around the world. To ensure that these medications are high quality and appropriately produced, countries require that animal health medicines are manufactured to specific standards of quality, with proven safety and efficacy. The responsible authority in a given country must authorize that a veterinary medicine can be manufactured, sold, and used. The marketing authorization, also known as "registration" or "license," implies that the responsible authority has approved not only the product to be marketed, but also the conditions that will characterize the use of the product. These conditions become part of the labelling, packaging, and information leaflets of the product and include (i) the characteristics of the active substance, its purity and concentration, and the complete composition of

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SCIENTIFIC OPINION

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ECDC, EFSA and EMA Joint Scientific Opinion on a list of outcome indicators as regards surveillance of antimicrobial resistance and antimicrobial consumption in humans and food-producing animals

ECDC, EFSA Panel on Biological Hazards (BIOHAZ) and
EMA Committee for Medicinal Products for Veterinary Use (CVMP)*

Abstract

ECDC, EFSA and EMA have jointly established a list of harmonised outcome indicators to assist EU Member States in assessing their progress in reducing the use of antimicrobials and antimicrobial resistance (AMR) in both humans and food-producing animals. The proposed indicators have been selected on the basis of data collected by Member States at the time of publication. For humans, the proposed indicators for antimicrobial consumption are: total consumption of antimicrobials (limited to antibacterials for systemic use), ratio of community consumption of certain classes of broad-spectrum to narrow-spectrum antimicrobials and consumption of selected broad-spectrum antimicrobials used in healthcare settings. The proposed indicators for AMR in humans are: meticillin-resistant *Staphylococcus aureus* and 3rd-generation cephalosporin-resistant *Escherichia coli*, *Klebsiella pneumoniae* resistant to aminoglycosides, fluoroquinolones and 3rd-generation cephalosporins, *Streptococcus pneumoniae* resistant to penicillin and *S. pneumoniae* resistant to macrolides, and *K. pneumoniae* resistant to carbapenems. For food-producing animals, indicators for antimicrobial consumption include: overall sales of veterinary antimicrobials, sales of 3rd- and 4th-generation cephalosporins, sales of quinolones and sales of polymyxins. Finally, proposed indicators for AMR in food-producing animals are: full susceptibility to a predefined panel of antimicrobials in *E. coli*, proportion of samples containing ESBL-/AmpC-producing *E. coli*, resistance to three or more antimicrobial classes in *E. coli* and resistance to ciprofloxacin in *E. coli*. For all sectors, the chosen indicators, which should be reconsidered at least every 5 years, are expected to be valid tools in monitoring antimicrobial consumption and AMR. With the exception of the proposed human AMR indicators, the indicators are in general not suitable to monitor the effects of targeted interventions in a specific sector, such as in a single animal species or animal production sector. Management decisions should never be based on these indicators alone but should take into account the underlying data and their analysis.

Keywords: antimicrobial consumption, antimicrobial resistance, food-producing animals, humans, indicator

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J. vet. Pharmacol. Therap. doi: 10.1111/jvp.12226 REVIEW ARTICLE

The management of risk arising from the use of antimicrobial agents in veterinary medicine in EU/EEA countries – a review

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Antimicrobials are essential medicines for the treatment of many microbial infections in humans and animals. Only a small number of antimicrobial agents with new mechanisms of action have been authorized in recent years for use in either humans or animals. Antimicrobial resistance (AMR) arising from the use of antimicrobial agents in veterinary medicine is a concern for public health due to the detection of increasing levels of resistance in foodborne zoonotic bacteria, particularly gram-negative bacteria, and due to the detection of determinants of resistance such as Extended-spectrum beta-lactamases (ESBL) in bacteria from animals and in foodstuffs of animal origin. The importance and the extent of the emergence and spread of AMR from animals to humans has yet to be quantified. Likewise, the relative contribution that the use of antimicrobial agents in animals makes to the overall risk to human from AMR is currently a subject of debate that can only be resolved through further research. Nevertheless, risk managers have agreed that the impact on public health of the use of antimicrobials in animals should be minimized as far as possible and a variety of measures have been introduced by different authorities in the EU to achieve this objective. This article reviews a range of measures that have been implemented within European countries to reduce the occurrence and the risk of transmission of AMR to humans following the use of antimicrobial agents in animals and briefly describes some of the alternatives to the use of antimicrobial agents that are being developed.

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The views expressed in this article are the personal views of the author(s) and may not be understood or quoted as being made on behalf of or reflecting the position of the European Medicines Agency or one of its committees or working parties.

INTRODUCTION

Antimicrobial resistance has been acknowledged as a potential consequence of the use of antimicrobial agents since the first days of the discovery of these compounds. In the time since Fleming's identification and isolation of penicillin in September 1928, we have learnt about these drugs and the threats against their effectiveness. Likewise concern is also now being increasingly focussed on the use of antimicrobial agents in veterinary medicine for the treatment of food-producing species, particularly as resistance levels in foodborne zoonotic bacteria are increasing (European Food Safety Authority/European Centre for Disease Prevention and Control, EFSA/ECDC, 2011, 2012). In the light of these figures, it is timely to reflect on the measures in place within the European Union/European Economic Area (EU/EAA) that are intended to reduce or contain the risks arising from the use of antimicrobial agents in veterinary medicines. This study reviews current risk management strategies that are applied within the EU/EAA to minimize this risk as a contribution to the current debate as to whether or not additional measures are required.

It is now apparent that the supply of new antimicrobial agents will be insufficient to replace those for which increased resistance levels have compromised their effectiveness. Therefore, the problem of AMR will remain for the foreseeable future. In addition, the number and spread of resistance genes

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Public health risk of antimicrobial resistance transfer from companion animals

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Antimicrobials are important tools for the therapy of infectious bacterial diseases in companion animals. Loss of efficacy of antimicrobial substances can seriously compromise animal health and welfare. A need for the development of new antimicrobials for the therapy of multiresistant infections, particularly those caused by Gram-negative bacteria, has been acknowledged in human medicine and a future corresponding need in veterinary medicine is expected. A unique aspect related to antimicrobial resistance and risk of resistance transfer in companion animals is their close contact with humans. This creates opportunities for interspecies transmission of resistant bacteria. Yet, the current knowledge of this field is limited and no risk assessment is performed when approving new veterinary antimicrobials. The objective of this review is to summarize the current knowledge on the use and indications for antimicrobials in companion animals, drug-resistant bacteria of concern among companion animals, risk factors for colonization of companion animals with resistant bacteria and transmission of antimicrobial resistance (bacteria and/or resistance determinants) between animals and humans. The major antimicrobial resistance microbiological hazards originating from companion animals that directly or indirectly may cause adverse health effects in humans are MRSA, methicillin-resistant *Staphylococcus pseudintermedius*, VRE, ESBL- or carbapenemase-producing Enterobacteriaceae and Gram-negative bacteria. In the face of the previously recognized microbiological hazards, a risk assessment tool could be applied in applications for marketing authorization for medicinal products for companion animals. This would allow the approval of new veterinary medicinal antimicrobials for which risk levels are estimated as acceptable for public health.

Introduction

During the last 50 years, the number of companion animals in modern society has substantially increased and a change in their social role has occurred. Attention to their welfare has increased as a consequence of the close contact between owners and their pets. Humans may acquire antimicrobial-resistant bacteria or the corresponding resistance genes not only from food-producing animals but also via contact with their companion animals.

MRSA, methicillin-resistant *Staphylococcus pseudintermedius* (MRSP), ESBL/AmpC-producing Enterobacteriaceae and MDR non-fermenting Gram-negative bacteria have emerged in healthy and sick dogs and cats, implying a potential risk of transmission of these bacteria to humans from infected or colonized companion animals.[1–3] In addition, there is the possibility of transfer of resistance genes.

In order to assess the risks within the context of applications for new veterinary antimicrobials for companion animals, there might be a need for additional data requirements with respect to antimicrobial resistance. The currently available guidance on pre-approval information for the registration of new veterinary medicinal products from the International Cooperation on Harmonisation of Technical Requirements for Registration of Veterinary Medicinal Products—VICH Topic GL27—is a guideline applicable to all new applications in the European, Japanese and

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Review

Use of colistin-containing products within the European Union and European Economic Area (EU/EEA): development of resistance in animals and possible impact on human and animal health

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Resistance

ABSTRACT

Since its introduction in the 1950s, colistin has been used mainly as a topical treatment in human medicine owing to its toxicity when given systemically. Sixty years later, colistin is being used as a last-resort drug to treat infections caused by multidrug-resistant (MDR) *Pseudomonas aeruginosa*, *Acinetobacter baumannii* and Enterobacteriaceae (e.g., *Escherichia coli*, *Klebsiella pneumoniae*), for which mortality can be high. In veterinary medicine, colistin has been used for decades for the treatment and prevention of infectious diseases. Colistin has been administered frequently as a group treatment for animal gastrointestinal infections caused by Gram-negative bacteria within intensive husbandry systems. Given the ever-growing need to retain the efficacy of antimicrobials used to treat MDR infections in humans, the use of colistin in veterinary medicine is being re-evaluated. Despite extensive use in veterinary medicine, there is limited evidence for the development of resistance to colistin and no evidence has been found for the transmission of resistance in bacteria that have been spread from animals to humans. Since surveillance for colistin resistance in animals is limited and the potential for such transmission exists, there is a clear

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Variations in the sales and sales patterns of veterinary antimicrobial agents in 25 European countries

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Objectives: To describe sales and sales patterns of veterinary antimicrobial agents in 25 European Union (EU)/European Economic Area (EEA) countries for 2011.

Methods: Data on the sales of veterinary antimicrobial agents from 25 EU member states and EEA countries for 2011 were collected at package level (name, formulation, strength, pack size, number of packages sold) according to a standardized protocol and template and presented in a harmonized manner. These data were calculated to express amounts sold, in metric tonnes, of active ingredient of each package. A population correction unit (PCU) was applied as a proxy for the animal biomass potentially treated with antimicrobial agents. The indicator used to express sales was milligrams of active substance per PCU.

Results: Substantial variations in the sales patterns and in the magnitude of sales of veterinary antimicrobial agents, expressed as mg/PCU, between the countries were observed. The proportion of sales, in mg/PCU, of products applicable for treatment of groups or herds of animals (premixes, oral powders and oral solution) varied considerably between the countries.

Conclusions: Some countries reported much lower sales of veterinary antimicrobial agents than others, when expressed as mg/PCU. Sales patterns varied between countries, particularly with respect to pharmaceutical forms. Further studies are needed to understand the factors that explain the observed differences.

Keywords: selection pressure, antimicrobial resistance, food safety, risk assessment, animal population, critically important antimicrobials

Introduction

Use of antimicrobial agents may promote the selection and dissemination of bacteria resistant to antimicrobials, and of resistance genes, as well as the emergence of new resistant bacteria through genetic mutations and gene movements. Antimicrobial resistance in bacteria causing disease in animals may limit therapeutic options and thereby have a direct impact on animal health and welfare. Furthermore, resistant bacteria and resistance genes may be disseminated from animals to humans through direct contact and via the food chain and the environment.[1,2] Use of antimicrobials for animals could thereby indirectly contribute to the increasing public health burden caused by antimicrobial resistance.[2]

In the European Union (EU)/European Economic Area (EEA) region, most antimicrobial classes that are marketed for use in animals are the same as or closely related to those classes used in human medicine. Important exceptions are the carbapenems, streptogramins, glycylcyclines, lipopeptides, oxazolidinones and glycopeptides, which are only authorized in human medicine. The use of antimicrobial agents in animals may select for resistant bacteria and resistance determinants of importance also in human medicine. Thus, antimicrobial stewardship in veterinary medicine and animal production is important for both animal and public health.

Data on the consumption, e.g. sales data and prescription data, of antimicrobial agents as well as data on resistance are essential components to inform policies and strategies for the containment of antimicrobial resistance. Such data are needed to measure the effect of e.g. campaigns promoting responsible use, adherence to guidelines and regulatory changes or to establish whether reduction targets are met. Furthermore, data on

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Review

Macrolides and lincosamides in cattle and pigs: Use and development of antimicrobial resistance

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ABSTRACT

Macrolides and lincosamides are important antibacterials for the treatment of many common infections in cattle and pigs. Products for in-feed medication with these compounds in combination with other antimicrobials are commonly used in Europe. Most recently approved injectable macrolides have very long elimination half-lives in both pigs and cattle, which allows once-only dosing regimens. Both in-feed medication and use of long-acting injections result in low concentrations of the active substance for prolonged periods, which causes concerns related to development of antimicrobial resistance.

Acquired resistance to macrolides and lincosamides among food animal pathogens, including some zoonotic bacteria, has now emerged. A comparison of studies on the prevalence of resistance is difficult, since for many micro-organisms no agreed standards for susceptibility testing are available. With animal pathogens, the most dramatic increase in resistance has been seen in the genus *Brachyspira*. Resistance towards macrolides and lincosamides has also been detected in staphylococci isolated from pigs and streptococci from cattle. This article reviews the use of macrolides and lincosamides in cattle and pigs, as well as the development of resistance in target and some zoonotic pathogens. The focus of the review is on European conditions.

Introduction

Macrolides are classified according to the number of atoms which comprise the lactone ring, ranging from 12 to 16 members (Yao and Moellering, 2007) (Table 1). The first macrolide intended for food animal use was spiramycin, which was introduced in the early 1960s, followed by erythromycin and tylosin in the early 1970s (Prescott, 2008). The most recent macrolide to be approved in the EU was tildipirosin in 2011. Semi-synthetic, new generation macrolides, the azalides, were introduced into human medicine in the early 1990s (Ballow and Amsden, 1992; Bryskier and Butzler, 2003). The first azalide for animal use, gamithromycin, was approved for use within the European Union (EU) in 2008.

Lincomycin and its semi-synthetic derivatives clindamycin and pirlimycin, belong to the lincosamides. In addition, streptogramins (A and B) are classified along with macrolides and lincosamides (Edelstein, 2004). The only streptogramin used for animals is

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[1] See: http://www.eucast.org/expert_rules/.

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Pleuromutilins: use in food-producing animals in the European Union, development of resistance and impact on human and animal health

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Pleuromutilins (tiamulin and valnemulin) are antimicrobial agents that are used mainly in veterinary medicine, especially for swine and to a lesser extent for poultry and rabbits. In pigs, tiamulin and valnemulin are used to treat swine dysentery, spirochaete-associated diarrhoea, porcine proliferative enteropathy, enzootic pneumonia and other infections where *Mycoplasma* is involved. There are concerns about the reported increases in the MICs of tiamulin and valnemulin for porcine *Brachyspira hyodysenteriae* isolates from different European countries, as only a limited number of antimicrobials are available for the treatment of swine dysentery where resistance to these antimicrobials is already common and widespread. The loss of pleuromutilins as effective tools to treat swine dysentery because of further increases in resistance or as a consequence of restrictions would present a considerable threat to pig health, welfare and productivity. In humans, only one product containing pleuromutilins (retapamulin) is authorized currently for topical use; however, products for oral and intravenous administration to humans with serious multidrug-resistant skin infections and respiratory infections, including those caused by methicillin-resistant *Staphylococcus aureus* (MRSA), are being developed. The objective of this review is to summarize the current knowledge on the usage of pleuromutilins, resistance development and the potential impact of this resistance on animal and human health.

Keywords: valnemulin, tiamulin, *Brachyspira hyodysenteriae*, review, antimicrobial resistance

Introduction

Pleuromutilin is a natural antimicrobial substance produced by the fungus *Pleurotus mutilus*, now called *Clitopilus scyphoides*.[1,2] Tiamulin and valnemulin are semi-synthetic derivatives of pleuromutilin and both drugs are used exclusively in veterinary medicine. Tiamulin was approved for use in veterinary medicine in 1979, followed by valnemulin in 1999.[3] Retapamulin was the first pleuromutilin approved for topical use for humans, in 2007.[4] A pleuromutilin for systemic use in humans, BC-3781, is currently under development.[3,5] Pleuromutilins are antibacterial agents that inhibit protein synthesis. They are active against Gram-positive bacteria such as streptococci and staphylococci, anaerobic bacteria and mycoplasmata; they have been used for decades in veterinary medicine for the control of respiratory and intestinal infections in different animal species, especially in pigs and to a lesser extent in poultry and rabbits.[6–8]

Use of pleuromutilins in veterinary medicine

Tiamulin is authorized nationally in the member states of the European Union (EU) and is available in most EU member states. Following a recent referral, tiamulin is indicated in pigs for the treatment and prevention of swine dysentery (*Brachyspira hyodysenteriae*), treatment of colitis (*Brachyspira pilosicoli*), treatment of ileitis (*Lawsonia intracellularis*) and treatment of enzootic pneumonia (*Mycoplasma hyopneumoniae*).[9] Other indications might still be listed, as different products containing tiamulin are nationally approved. Tiamulin is also authorized for chickens for the treatment and prevention of chronic respiratory disease and air-sacculitis caused by *Mycoplasma gallisepticum* and *Mycoplasma synoviae*; for turkeys for the treatment and prevention of infectious sinusitis and air-sacculitis caused by *M. gallisepticum*, *Mycoplasma meleagridis* and *M. synoviae*; and for rabbits for the treatment of epizootic rabbit enterocolitis. Valnemulin is

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Comparison of the sales of veterinary antibacterial agents between 10 European countries

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Objectives: To compare the sales of veterinary antibacterial agents between 10 European countries.

Methods: Data were compiled from published reports from the 10 countries. We express the usage as amounts sold of veterinary antibacterial agents per country and year, in mg active substances per kg biomass of slaughtered pigs, poultry and cattle plus estimated biomass of (live) dairy cattle for the corresponding year.

Results: The usage, as expressed in mg antibacterial drugs sold/kg biomass of slaughtered pigs, poultry and cattle and of (live) dairy cattle, varied from 18 to 188 mg/kg. The relative proportion of the various classes of antibacterial agents sold varied considerably.

Conclusions: The apparent wide variations in the usage of veterinary antimicrobial agents between countries cannot be explained by differences in the animal species demographics alone. Further in-depth analyses are required to identify the factors underlying the observed differences.

Keywords: risk factors, antimicrobial resistance, food safety, risk assessment

Introduction

Data generated from surveillance of the usage of veterinary antibacterial agents are essential to identify and quantify risk factors for the development and occurrence of resistance, such as methicillin-resistant *Staphylococcus aureus* (MRSA) or extended-spectrum β-lactamase (ESBL)-producing bacteria[1] in animals, as well as its impact on human health. Data on the use of antibacterial agents can be combined with data on antimicrobial resistance to inform the development of policies for the containment of antimicrobial resistance at national, regional and international levels.[2]

This article compares and discusses the usage and usage patterns of veterinary antibacterial agents between 10 European countries and is a first step towards the collation of data on usage of antibacterial agents categorized by animal species at the European level.

Methods

Currently, data on overall sales of veterinary antibacterial agents are published on a regular basis by 10 European countries: the Czech Republic; Denmark; Finland; France; Germany; the Netherlands; Norway; Sweden; Switzerland; and the UK.[3–12] We have compiled overall sales data of veterinary antibacterial agents for therapeutic use from the published reports into a standardized table format; the data represent sales in 2007 except for Germany for which the most recent figures were for 2005.

The sales figures are reported in tons of active substance sold per antibacterial class (e.g. tetracyclines).

The overall sales data generally represent sales for use in the total animal population in the various countries in 2007. However, the inclusion criteria of veterinary antibacterial agents were inadequately described or lacking in several of the reports; therefore the data coverage may vary and affect its interpretation.

As the majority of antibacterial veterinary products are marketed for more than one species the sales cannot be ascribed to a single target species and thus the sales cannot be reported relative to the size of the target population, e.g. per number of slaughter pigs produced per study year and country. To correct for the population 'at risk' of being treated, a pragmatic approach is to use the total biomass of major production animals as the denominator.[13] We derived data for slaughtered pigs, poultry and cattle and of (live) dairy cattle for the various Member States from the Eurostat[14] database while for Norway and Switzerland data from Statistics Norway[15] and Swiss Statistics[16] were applied. We calculated the biomass of live dairy cattle by multiplying numbers of dairy cattle by the standard average weight of the various breeds (500 kg).

The type and incidence of bacterial diseases vary considerably between species and consequently the consumption of veterinary antibacterial agents is heavily influenced by animal species demographics. As the biomass produced reflects the population, we applied the above-described denominator as a measure to express the differences in the population patterns.

In the current paper, the usage is expressed as amounts sold, in mg active substances, of veterinary antibacterial agents per the total