SCIENTIFIC REPORT

ECDC/EFSA/EMA second joint report on the integrated analysis of the consumption of antimicrobial agents and occurrence of antimicrobial resistance in bacteria from humans and food-producing animals

Joint Interagency Antimicrobial Consumption and Resistance Analysis (JIACRA) Report

European Centre for Disease Prevention and Control (ECDC),
European Food Safety Authority (EFSA) and
European Medicines Agency (EMA)

Abstract

The second ECDC/EFSA/EMA joint report on the integrated analysis of antimicrobial consumption (AMC) and antimicrobial resistance (AMR) in bacteria from humans and food-producing animals addressed data obtained by the Agencies' EU-wide surveillance networks for 2013–2015. AMC in both sectors, expressed in mg/kg of estimated biomass, were compared at country and European level. Substantial variations between countries were observed in both sectors. Estimated data on AMC for pigs and poultry were used for the first time. Univariate and multivariate analyses were applied to study associations between AMC and AMR. In 2014, the average AMC was higher in animals (152 mg/kg) than in humans (124 mg/kg), but the opposite applied to the median AMC (67 and 118 mg/kg, respectively). In 18 of 28 countries, AMC was lower in animals than in humans. Univariate analysis showed statistically-significant ($p < 0.05$) associations between AMC and AMR for fluoroquinolones and *Escherichia coli* in both sectors, for 3rd- and 4th-generation cephalosporins and *E. coli* in humans, and tetracyclines and polymyxins and *E. coli* in animals. In humans, there was a statistically-significant association between AMC and AMR for carbapenems and polymyxins in *Klebsiella pneumoniae.* Consumption of macrolides in animals was significantly associated with macrolide resistance in *Campylobacter coli* in animals and humans. Multivariate analyses provided a unique approach to assess the contributions of AMC in humans and animals and AMR in bacteria from animals to AMR in bacteria from humans. Multivariate analyses demonstrated that 3rd- and 4th-generation cephalosporin and fluoroquinolone resistance in *E. coli* from humans was associated with corresponding AMC in humans, whereas resistance to fluoroquinolones in *Salmonella* spp. and *Campylobacter* spp. from humans was related to consumption of fluoroquinolones in animals. These results suggest that from a 'One-health' perspective, there is potential in both sectors to further develop prudent use of antimicrobials and thereby reduce AMR.

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Procedures for adoption of the report: Representatives of the different surveillance/monitoring networks of the MSs in charge of providing the data and the European Union Reference Laboratory for Antimicrobial Resistance (EURL-AR) have been consulted at the finalisation of the preparation of the joint report. ECDC, EFSA and EMA have each established their own procedure for endorsement or adoption of the joint report according to their internal rules.
ECDC adopted the report on 28 June 2017, after consultation with the European Antimicrobial Resistance Surveillance Network (EARS-Net), the European Surveillance Antimicrobial Consumption Network (ESAC-Net), the Food- and Waterborne Diseases and Zoonoses Network (FWD-Net), and the ECDC Advisory Forum. EFSA approved the report on 28 June 2017, after consultation of its Scientific Network for Zoonosis Monitoring Data. EMA adopted the report on 28 June 2017. Before endorsement the report was circulated for consultation to the European Surveillance of Veterinary Antimicrobial Consumption (ESVAC) network. The report was circulated on 4 July 2017 to the CVMP plenary meeting for information.

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Summary

This report on integrated analyses of potential relationships between the consumption of antimicrobial agents (AMC) and the occurrence bacterial of antimicrobial resistance (AMR) in humans and in food-producing animals was produced by the European Centre for Disease Prevention and Control (ECDC), the European Food Safety Authority (EFSA) and the European Medicines Agency (EMA). This is the second report of its kind prepared by the three agencies following a request from the European Commission (EC). The data included in the analyses and the results presented in the report pertain to 2013, 2014 and 2015 and were obtained through five different surveillance/monitoring networks managed by the agencies to collect annual data on AMC and AMR in humans and in food-producing animals in the EU/EEA Member States (MSs) and in Switzerland. The data used in the analyses were collected as part of clinical and epidemiological surveillance/monitoring in both humans and food-producing animals, and were not collected specifically for the purpose of this report. The integrated analyses of data from humans and animals presented here were based on the 'One-Health' approach and focused on particular combinations of antimicrobials and bacterial species considered of importance for public health.

Compared to the first JIACRA report, the number of countries reporting AMC data increased in both humans (30, previously 28) and food-producing animals (29, previously 26). AMC data from humans are commonly reported as defined daily doses (DDD) per 1,000 inhabitants and per day, whereas the corresponding data in food-producing animals are typically expressed in milligrams of active substance per kilogram of estimated biomass and per year. As a fully comparable measurement unit is not available, AMC data in humans were converted into mass of active substance per kilogram of estimated biomass to make the comparison as consistent as possible.

To facilitate the comparison of AMC and AMR and for the sake of consistency with the first JIACRA report, a summary indicator of microbiological resistance (SIMR) was calculated for food-producing animals on the basis of the weighted mean by estimated biomass of the proportions of drug-resistant bacteria in each animal species included in the indicator. For data referring to 2013, the SIMR in bacteria from food-producing animals addressed AMR data covering cattle, pigs and broilers, whereas, for data referring to 2014–2015, AMR data on broilers, fattening turkeys, fattening pigs and cattle under 1 year of age were included. An additional SIMR in bacteria from poultry was also constructed based on AMR data on broilers and turkeys for 2014–2015. These differences reflect changes in the EU legislation on AMR monitoring in food-producing animals implemented in 2014. SIMR were compared to corresponding AMC in food-producing animals.

To date, AMC data in food-producing animals are not available by species in the European Surveillance of Veterinary Antimicrobial Consumption (ESVAC) reports, whereas, in this report, an attempt to infer AMC at the animal species level was made. Technically derived estimates of sales of antimicrobials in pigs and poultry were used as a proxy for the exposure to antimicrobials (DDDvet/kg of estimated biomass and year) in a species or species group (poultry). This proxy was subsequently used to investigate potential associations between AMC and the occurrence of AMR at species or species group (poultry) level. Although AMR data also has covered bovine animals under 1 year of age from 2015 onwards, AMC data could not be inferred at the level of this subpopulation, because cattle in general is typically given as the target species in the summaries of product characteristics (SPCs).

To analyse the relationship between AMC and AMR in humans and food-producing animals, both univariate analysis (logistic regression) and multivariate analysis (Partial Least Squares Path Modeling) were employed. Logistic regression was used to assess the associations between (1) AMC in humans and occurrence of AMR in isolates from humans, (2) AMC in food-producing animals and occurrence of AMR in isolates from food-producing animals, (3) occurrence of AMR in isolates from food-producing animals and occurrence of AMR in isolates from humans, and (4) AMC in food-producing animals and occurrence of AMR in isolates from humans, for the years 2013–2015. To further assess potential relationships between AMR in isolates from humans and AMC in humans (in the community and at the hospital), AMC in food-producing animals (pigs and poultry), and AMR in isolates from food-producing animals (pigs and poultry), in a simultaneous manner, multivariate analyses were also performed. The analyses were made per antimicrobial class. Two approaches were used to assess further relationships between AMC and AMR, while accounting for the phenomenon of co-selection.

In 2014, the average total consumption of antimicrobials expressed in milligrams of active substance per kilogram of estimated biomass was 124 mg/kg in humans (median 118 mg/kg; range 50–182 mg/kg) and 152 mg/kg in food-producing animals (median 67 mg/kg; range 3–419). AMC was lower or much lower in food-producing animals than in humans in 18 of 28 countries included in the

analysis, in two countries, AMC was similar, and in the eight remaining countries, AMC was higher or much higher in food-producing animals than in humans. These observations are similar to those reported in the first JIACRA report (ECDC/EFSA/EMA, 2015).

The consumption of 3rd- and 4th-generation cephalosporins occurred predominantly in human medicine and particularly in hospitals, with a much lower consumption in food-producing animals. The consumption in humans was significantly associated with resistance to 3rd-generation cephalosporins of invasive *Escherichia coli* from humans. For *Salmonella* spp., the situation was different, with few statistically-significant positive associations between consumption of 3rd- and 4th-generation cephalosporins and resistance to these antimicrobial agents. Statistically-significant associations were observed between the total consumption of 3rd- and 4th-generation cephalosporins in food-producing animals and the SIMR to 3rd-generation cephalosporins in *E. coli* and *Salmonella* spp. from food-producing animals in 2014–2015 but in this case, the statistical significance was lost on removal of an outlying value. Statistically-significant positive associations were found between occurrence of resistance to 3rd-generation cephalosporins in indicator *E. coli* from food-producing animals and the occurrence of resistance in invasive *E. coli* from humans, but only when considering the aggregated resistance data on food-producing animals (SIMR) in 2014–2015 and after removing outliers. No statistically-significant associations were observed between the consumption of 3rd- and 4th-generation cephalosporins in food-producing animals and the occurrence of resistance to 3rd-generation cephalosporins in selected bacteria from humans. The multivariate analyses showed that for resistance to 3rd-generation cephalosporins in invasive *E. coli* from humans, there was a significant direct effect of the consumption of 3rd- and 4th-generation cephalosporins in humans, and hospital consumption appeared to have the greatest weight, but there was no statistically-significant effect of the consumption of 3rd- and 4th-generation cephalosporin in food-producing animals or the corresponding resistance in commensal *E. coli* from food-producing animals. Associations between AMC and AMR were assessed using available data on the occurrence of phenotypic resistance to 3rd- and 4th-generation cephalosporins. Investigation of these associations in specific animal sub-populations, and accounting for resistance genotypes would have helped in further refining the analysis, but could not be performed because of lack of data.

For fluoroquinolones, consumption was overall lower in food-producing animals than in humans in most countries, but the difference in consumption was less marked than that of the 3rd- and 4th-generation cephalosporins. Consumption of fluoroquinolones in humans, which mostly occurs in the community, was significantly associated with resistance to fluoroquinolones in invasive *E. coli* from humans, but not with fluoroquinolone resistance in *Salmonella* spp. or *Campylobacter* spp. from humans. Statistically-significant positive associations between the consumption of fluoroquinolones and other quinolones in food-producing animals and resistance to fluoroquinolones in indicator *E. coli*, *Salmonella* spp., *C. jejuni* and *C. coli* from such animals were observed for 2013, 2014 and 2015. When data on pigs and poultry were analysed separately, a statistically-significant association was not consistently observed in pigs (*E. coli*) or poultry (*Salmonella* spp.). Statistically-significant associations were observed between total consumption of fluoroquinolones and other quinolones in food-producing animals and the occurrence of resistance to fluoroquinolones in invasive *E. coli* from humans. Statistically-significant associations were observed between the occurrence of resistance to fluoroquinolones in *E. coli*, *C. jejuni* and *C. coli* from food-producing animals (poultry in the case of *C. jejuni* isolates) and the occurrence of resistance in *E. coli*, *C. jejuni* and *C. coli* from human infections; no such statistically-significant association was observed for *Salmonella* spp. Multivariate analyses showed that resistance to fluoroquinolones and other quinolones in *Salmonella* spp. and *C. jejuni* from humans was significantly associated with resistance in bacteria from food-producing animals, which, in turn, was significantly associated to the consumption of fluoroquinolones and quinolones in food-producing animals. No such statistically-significant association between resistance to fluoroquinolones of invasive *E. coli* from humans and fluoroquinolone consumption or fluoroquinolone resistance in indicator *E. coli* in food-producing animals was observed in the multivariate analysis.

For polymyxins (colistin), the consumption in food-producing animals by far outweighed that reported in humans. Large variations were observed in the quantities of polymyxins consumed in food-producing animals by country, whereas a few countries did not use polymyxins in food-producing animals. In humans, statistically-significant positive associations were observed between the total consumption and the hospital consumption, of polymyxins and resistance to polymyxins in invasive *Klebsiella pneumoniae*, whereas there was no statistically-significant association between the consumption of polymyxins in the community and the corresponding resistance in *K. pneumoniae*.

Statistically-significant positive associations were observed between polymyxin consumption in food-producing animals and the corresponding SIMR in indicator *E. coli* from food-producing animals, from poultry and from pigs, even though the occurrence of resistance to polymyxins in animals is typically low. The structural lack of comparability of the available data on polymyxins (colistin) in humans and food-producing animals did not allow fitting a multivariate analysis.

Consumption of macrolides was overall similar in food-producing animals and in humans, although the quantities consumed varied between countries. In humans, macrolides are nearly entirely consumed in the community. No statistically-significant associations were observed between the consumption of macrolides in humans and the corresponding resistance to macrolides in *Campylobacter* spp. from humans. A statistically-significant positive association was observed between macrolide consumption in food-producing animals and resistance to macrolides in *C. coli* from food-producing animals for the year 2014–2015, but not in *C. jejuni* for the same period. The data available for this analysis were sparse. Statistically-significant associations were noted for consumption of macrolides in food-producing animals and the occurrence of resistance to macrolides in *C. jejuni* and *C. coli* from humans, whereas resistance to macrolides in *Campylobacter* spp. isolates from food-producing animals was significantly associated with resistance in humans for *C. coli* from broilers but not for *C. jejuni* from poultry. Data were insufficient to allow a multivariate analysis to be performed.

In most countries, the amount of tetracyclines consumed in food-producing animals markedly outweighed that consumed in humans, with large variations in animal consumption between countries. Few statistically-significant associations were observed between consumption of tetracyclines in humans and resistance to tetracyclines in *Salmonella* spp. and *Campylobacter* spp. from humans. Statistically-significant positive associations were observed between tetracycline consumption in food-producing animals and resistance to tetracyclines in indicator *E. coli, Salmonella* spp. and *C. jejuni* from food-producing animals for 2013, 2014 and 2015. The associations remained statistically-significant for indicator *E. coli* when data for pigs and poultry were analysed separately as it did for *C. jejuni* from poultry, although not for *Salmonella* spp. from poultry. Multivariate analyses showed that resistance to tetracyclines in *C. jejuni* from humans was significantly associated with resistance to tetracyclines in bacteria from food-producing animals, which, in turn, was significantly associated with the consumption of tetracyclines in food-producing animals.

Carbapenems are only authorised for use in humans and consumption of carbapenems in humans was significantly associated with resistance to carbapenems in invasive *K. pneumoniae* from humans. In food-producing animals, there were only very rare single isolates of *E. coli* and *Salmonella* spp. reported as resistant to carbapenems during the study period and therefore, no further analysis could be carried out.

The reporting of AMR data at the individual isolate level in the animal sector also allowed for characterisation of phenotypic profiles of resistance to the harmonised panel of antimicrobial substances tested. This enabled analysis of complete susceptibility, defined as susceptibility to all the antimicrobial classes tested of the harmonised panel. In food-producing animals, a statistically-significant negative association was consistently detected between the total AMC and the occurrence of complete susceptibility. This suggests that measures aimed at encouraging prudent use should concern all antimicrobial classes consumed so as to take into account the potential impact of the phenomenon of co-selection of AMR. Because of the wide range in total AMC and in the occurrence of complete susceptibility in bacteria from food-producing animals in different MSs, this parameter might be considered as a potential candidate for an epidemiological indicator for animals.

The reporting of AMR isolate-based data allowed for taking into account the phenomenon of co-selection in investigating potential associations between AMC and AMR in indicator *E. coli* in food-producing animals. The associations already observed as statistically-significant in the univariate analyses were confirmed with the confidence intervals of odds ratios narrowed, and two new associations (2013, tetracyclines/indicator *E. coli*; 2013, 3rd- and 4th-generation cephalosporins/ indicator *E. coli*) were newly discerned as statistically-significant. The results illustrated the potential value of accounting for co-selection when assessing relationships between AMC and AMR.

Many of the observed findings fit well with the current knowledge of the epidemiology of AMR and infection relating to the bacterial species studied. In food-producing animals, stronger and more consistent associations between AMC and AMR were more frequently observed for indicator *E. coli* than for *Salmonella* spp. Similar observations were reported in the first JIACRA report (ECDC/EFSA/EMA, 2015). The multivariate analyses also illustrated that, depending on the bacteria/antimicrobial class combinations considered, the relative strength of the associations between AMC and AMR within the human sector and between AMC in animals and AMR in bacteria from humans, differed

markedly. Overall, this report confirms the positive association between AMC and AMR in both humans and food-producing animals and underlines the need to ensure prudent use so as to reduce the consumption of antimicrobials in both food-producing animals and humans (Official Journal of the European Union, 2015; EMA/CVMP, 2016; EMA/EFSA, 2017; ECDC, 2017b).

The epidemiology of resistance is complex and factors other than the amount of antimicrobials consumed may influence the level of resistance. The bacterial species studied include some in which spread of drug-resistant clones is a highly important feature. This may impact on the assessment between AMC and AMR and could explain why significant statistical associations between antimicrobial usage and resistance were detected in some cases, and not in others, e.g. regarding *K. pneumoniae* and also in some cases for *Salmonella* spp. and certain salmonella serovars.

This report makes use of data collected in different surveillance/monitoring systems with different aims and primary purposes in each sector. The integrated analysis of such data is inherently hindered by this feature, and the intrinsic characteristics of both the monitoring data used and the analysis approaches applied should be considered when assessing the results. The availability of more detailed and comprehensive data would increase the scope of the analyses that can be performed and improve the robustness of the outputs. Other factors that could be considered in the analysis are resistance to other antimicrobials (co-resistance), travel by humans, transfer of patients between hospitals, import and trade of food, food of non-animal origin, trade of live animals both between and within countries and exposure of animals or humans *via* the environment. AMC data should preferably be collected so that analysis of its component parts is possible (hospital vs community antimicrobial usage or usage in pigs vs cattle vs broilers vs turkeys). The more complete availability of AMC data by animal species expressed in numbers of DDDvet should help in addressing this. Additional information, including AMC by animal species and collection of AMR data from all countries, from relevant animal species, at a detailed level, including production type, is required to further improve the analysis performed.

A possible specific approach to acquiring necessary data would comprise dedicated studies or surveys specifically to collect data from the two sectors for integrated analysis. Examples of the use of such specific methodology include the EU-wide baseline studies on the prevalence of *Salmonella* spp. for animals and the point prevalence surveys of healthcare-associated infections for humans. Data on the occurrence of AMR in *E. coli* from healthy humans would probably be a good indicator of the relative exposure to antimicrobial-resistant bacteria through food consumption and the direct effect of AMC in the community on bacteria in humans. The ability to compare commensal *E. coli* in humans and food-producing animals might be particularly useful in a multivariate analysis approach.

The multivariate analysis performed proved to have strong potential to support conclusions in a 'One Health'-perspective and should be further used. The findings of ecological analyses such as those presented in this report should be considered as hypotheses for subsequent testing by focused research that in time could provide better explanations for the observed associations, where these are lacking.

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1.   Introduction

Following a request from the European Commission (EC) in 2015 the European Centre for Disease Prevention and Control (ECDC), the European Food Safety Authority (EFSA) and the European Medicines Agency (EMA) published the first Joint Inter-agency Antimicrobial Consumption and Resistance Analysis (JIACRA) Report (ECDC/EFSA/EMA, 2015). The first JIACRA report provided, for the first time, an analysis of possible relationships between the consumption of antimicrobial agents in human and veterinary medicine and the occurrence of antimicrobial resistance (AMR) in bacteria from humans and food-producing animals at the European level (Appendices A and C). The request was based on the Communication of 15 November 2011 from the Commission to the European Parliament and the Council: 'Action Plan against the rising threats from Antimicrobial Resistance' (European Commission, 2011), which sets out key actions of the Commission addressing the problem of AMR. It was the first such integrated analysis worldwide considering a broad range of data from the food-producing animal sector and humans.

In December 2015, the EC requested ECDC, EFSA and EMA to continue their analysis of data and to publish a second JIACRA report based on data from relevant European Union (EU) surveillance systems for the years 2013 and 2014, to be completed by the end of 2016. Following a request from the three agencies submitted in January 2016, in March 2016, the EC agreed to extend the completion date of the report to the end of June 2017, and to include available data from 2015 as well as 2013 and 2014. The second JIACRA report benefits from refinements and improvements to the surveillance performed by the different networks, i.e. implementation of new legislation on harmonised resistance monitoring in animals undertaken by EFSA, EMA work on establishment of defined daily doses for animals (DDDvet) values, the increased number of participating Member States (MSs) and improved harmonisation of surveillance of AMR in foodborne pathogens in humans undertaken by ECDC. This has allowed use of enhanced methodological approaches to the analysis of the data compared to the first JIACRA report.

2.   Terms of reference and scope

The aim of this second report is, as for the first JIACRA Report, to provide an integrated analysis of relationships between the consumption of antimicrobial agents in human and veterinary medicine and the occurrence of AMR in bacteria from humans and food-producing animals. The data originate from five different EU-wide surveillance systems run by the three agencies. For this report, ECDC has provided data on AMC in humans as well as data on AMR in isolates from cases of human infection. The EFSA has provided data on AMR in bacteria from food-producing animals. The EMA has provided data on AMC in food-producing animals. All the data collected by the networks were originally provided by the countries listed in the respective original reports.

The scope of the report is limited to a comparison of consumption of antimicrobials in food-producing animals and humans and to the analysis of the prevalence of resistance to certain antimicrobials in selected bacteria; it includes consideration of co-resistance and completely-susceptible bacteria. The analysis of trends in resistance and consumption is presented already in the annual reports of the three agencies (ECDC, 2014b, 2016c, 2017a; EMA/ESVAC, 2016b; EFSA/ECDC, 2017). This information is therefore not included in the second JIACRA report, which primarily investigated statistical associations between AMC and AMR.

3.   Brief outline of surveillance systems providing data for this report

ECDC has a mandate to gather and analyse data and information on emerging public health threats and developments for the purpose of protecting public health in the European Union according to Regulation 851/2004/EC (Official Journal of the European Union, 2004). Data included in this report regarding the occurrence of resistance in humans were obtained from two surveillance networks – the European Antimicrobial Resistance Surveillance Network (EARS-Net) and the Food- and Waterborne Diseases and Zoonoses Network (FWD-Net). Data regarding consumption of antimicrobials in humans were obtained from the European Surveillance of Antimicrobial Consumption Network (ESAC-Net).

Based on Article 33 in Regulation (EC) 178/2002 (Official Journal of the European Communities, 2002), EFSA is responsible for examining data on zoonoses, AMR and foodborne outbreaks collected from the MSs in accordance with Directive 2003/99/EC (Official Journal of the European Union, 2003) and for preparing the EU Summary Report from the results. Regarding AMR data, a specific EU Summary Report on AMR is produced in collaboration with ECDC on a yearly basis. The EU Summary Report on AMR includes data related to the occurrence of AMR both in isolates from animals and

foodstuffs, collected in the framework of Directive 2003/99/EC (Official Journal of the European Union, 2003), and in isolates from human cases, derived from FWD-Net, coordinated by ECDC.

The main responsibility of the EMA is the protection and promotion of public and animal health, through the evaluation and supervision of medicines for human and veterinary use. The ESVAC project was launched by the agency in September 2009, following a request from the EC to develop a harmonised approach to the collection and reporting of data on the consumption of antimicrobial agents in animals from the MSs. The ESVAC reports present data on the consumption of veterinary antimicrobial agents from EU and European Economic Area (EEA) countries, provided at package level according to a standardised protocol and template.

3.1. Surveillance of antimicrobial consumption in humans

3.1.1. Description of collected data

ESAC-Net is the continuation of the former ESAC project and is a Europe-wide network of national surveillance systems coordinated by ECDC providing independent reference data on AMC in all EU MSs, as well in the EEA countries, Iceland and Norway. It collects and analyses AMC data from the community (primary care) and from hospitals.

Antimicrobials are grouped according to the anatomical therapeutic chemical (ATC) classification. The three major categories of antimicrobials considered in ESAC-Net are the antibacterials for systemic use (ATC group J01), antimycotics and antifungals (J02 and D01BA), and antivirals (J05). Only antimicrobials that are 'antibacterials for systemic use' (ATC J01) are included in the present report.

There are two options for reporting ESAC-Net data to ECDC:

- the preferred standard option, i.e. reporting of national AMC data at the medicinal product level, expressed as number of packages sold or reimbursed. For this option, a valid national registry of available antimicrobials is required (national registry data);
- a 'light' version, e.g. when national registry data are not available, reporting of aggregated numbers of DDD (defined daily doses) per 1,000 inhabitants and per day from national AMC data at the ATC substance level.

In addition, ESAC-Net encourages participants to report data by age group, gender and type of prescriber, as well as to report annually AMC data stratified by quarter rather than by year.

Most countries report data on sales, one-third of the countries report reimbursement data and a few report both sales and reimbursement data.

Data are uploaded into the Epidemiological Surveillance System (TESSy) database and used for reporting after a validation process and final approval by national ECDC contact points nominated by the reporting countries. The reporting countries can at any time upload or re-upload data to TESSy, e.g. for correction purposes. For the current report, the data used represents the data uploaded in TESSy on 1 February 2017.

ECDC ensures the annual analysis of the trends in overall AMC and by the different ATC groups, as well as comparisons between countries. Public access to information on AMC in Europe is provided through an ESAC-Net interactive database and an ECDC EU summary report on AMC.

3.1.2. Strength of the system

The ESAC-Net collects data from 30 EU/EEA MSs. For most of these countries, complete national consumption was reported. The quality of AMC data also depends on the type of data available for a given sector. For most of the countries, ESAC-Net can differentiate between AMC data from the community (primary care) and from hospitals.

Data provided through the standard option of the reporting protocol are very valuable. First, the level of detail of these data (complete registry of products) allows a better quality check of the provided consumption data. Second, it provides the opportunity to carry out studies on the number of packages consumed to estimate the number of prescriptions or detailed analyses (such as analyses on the availability of products or changes in the content of products).

A standardised reporting protocol is essential to ensure comparability with data obtained by other multinational surveillance networks. The WHO Regional Office for Europe established an AMC network of 18 southern and eastern European countries. WHO Headquarters recently developed a simplified

AMC surveillance protocol within the framework of the WHO Global Action Plan on AMR (WHO, 2016). The surveillance protocols are aligned to the ESAC-Net protocol and may also help to generate comparable AMC data from outside the EU in the future.

3.1.3. Impediments to comparing the data

For ESAC-Net, countries provide sales and/or reimbursement data that each have limitations. The major limitation of reimbursement data is that they do not include antimicrobials dispensed without a prescription and non-reimbursed prescribed antimicrobials (for example, the antimicrobials prescribed through private healthcare systems).

Countries, from one year to another, might deliver different types of data or report from different data sources, which could also introduce bias in the consumption rates reported. The number of countries that each year change data provider and/or types of data is small.

ESAC-Net reports consumption separately for the community and the hospital sector, but some countries that are not able to split data according to the healthcare sector reported totals from both sectors combined (total care). Because the overall consumption in the community has been shown to represents around 90% of the total consumption (when expressed in DDD per 1,000 inhabitants and per day and reported for the ATC group J01), ESAC-Net reports the total care consumption as community consumption for those countries not able to split the data. For these countries, the figures reported for the community are overestimated and the antimicrobials normally used in the hospitals will be reported in the community sector; thus, the pattern of consumption will be slightly different from that seen in countries providing separate data for the community and hospitals.

Although all countries are able to report AMC for the community, over 2013–2015, five countries could not report data for the hospital sector as there was no surveillance system in place to collect data from this sector.

Finally, ESAC-Net reports the hospital consumption using the whole population as denominator and not hospital activity indicators, such as the number of admissions or patient bed days, which may not be completely comparable in terms of trends.

3.1.4. On-going actions to improve the system

To improve the reporting of hospital AMC, ESAC-Net is developing a hospital-based surveillance of AMC. This surveillance will enable countries not currently reporting data for the hospital sector to do so in the future. In addition, consumption data will be collected by type of hospital as well as by hospital activity indicator in order to relate consumption to actual hospital activity.

ESAC-Net aims to comply with ECDC's long-term surveillance strategy for 2014–2020, which targets improved routine surveillance outputs. It includes reusable online content of the ESAC-Net database (public ECDC Atlas for Infectious Diseases), which could replace large parts of the lengthy surveillance reports. These reports will, in turn, be shorter and focus more on data interpretation relevant to public health.

3.2. Surveillance of antimicrobial consumption in food-producing animals

3.2.1. Description of collected data

The ESVAC project coordinated by EMA collects harmonised data on overall sales of antimicrobial veterinary medicinal products (VMPs) at package level from most of the EU MSs and also from Iceland, Norway and Switzerland. The sales data are collected from various national sources (wholesalers, marketing authorisation holders (MAHs), feed mills and pharmacies) and the data presented by antimicrobial class or sub-class according to the anatomical therapeutic chemical animals (ATCvet) classification system.

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 data base, e.g. for correction purposes. For the current report, the data used for the calculation of estimated consumption by species represents the data available in the ESVAC data base on 1 February 2017. The data describing the overall consumption represents the datasets uploaded in the ESVAC database by 1 December 2016.

In the analysis of data, products formulated as tablets, which are almost exclusively used for companion animals, are reported separately. The remaining products are mainly used for food-producing animals and data on these products are used for the analyses presented in the current report.

In order to normalise the sales data for the animal population that can be subjected to treatment with antimicrobial agents, a population correction unit (PCU) is used as a proxy for the size of the animal population at risk of being treated. The PCU is purely a technical unit of measurement, used only to estimate sales corrected by the animal population in the individual countries; 1 PCU = 1 kg of different categories of livestock and slaughtered animals. The data sources used and the methodology for the calculation of PCU are comprehensively described in Appendix 2 to EMA's report 'Trends in the sales of veterinary antimicrobial agents in nine European countries: 2005–2009' (EMA/ESVAC, 2011).

The main indicator applied in this report to express the overall sales and by class/subclass of veterinary antimicrobials is mg active ingredient normalised by the population correction unit (mg/PCU): amount sold in tonnes $\times$ 10^9 divided by PCU in kg.

Because the VMPs are typically marketed for more than one species, the sales data as such do not provide information on sales by animal species. Therefore, technical derived estimates have been calculated for pigs and poultry for the purpose of this report (see Section 4.4).

3.2.2. Strength of the system

The ESVAC sales data covers 26 EU MSs and two EEA countries for 2013 while for 2014 and 2015 this figure is 29, including Switzerland and covers also data from animal hospitals. The standardised ESVAC reporting protocol and data collection template is essential to ensure that harmonised data are collected from the participating countries. The web-based delivery of the data to the ESVAC sales database ensures that these are standardised and the ESVAC-Business Intelligence (BI) ensures that data are calculated in a standardised manner from number of packages to weight of active substance in accordance with the ESVAC sales protocol. To support validation of the data by EMA as well as the MSs, standard reports designed for validation are produced by the ESVAC-BI.

3.2.3. Impediments to comparing data

The national consumption data for antimicrobial agents (numerator) cover all food-producing animal species, including horses. This means that the animal population 'at risk' of being treated with antimicrobial agents (denominator) includes all food-producing species. The consumption of antimicrobial agents by the various animal species varies considerably. For example, the consumption of antimicrobial agents in extensively reared sheep and goats is generally low, while consumption in intensively reared calves can be substantial. Therefore, the interpretation of these data should take into account the distribution of the PCU value between the species in the various countries. It should also be emphasised again that the PCU only represents a technical unit of measurement and not a real value for the animal population that could potentially be treated by antimicrobial agents.

3.2.4. On-going actions to improve the system

Discussions with the ESVAC participating countries to improve the quality of the data are taking place continuously, primarily through the validation process as described in Section 3.2.2 but also during the annual network meetings. Further details on the evolution of the ESVAC activity are provided on the document ESVAC: Vision, Strategy and Objectives 2016–2020 (EMA/326299/2015) (EMA/ESVAC, 2016a).

3.3. Surveillance of antimicrobial resistance in humans

3.3.1. Surveillance of antimicrobial resistance in bacteria from humans through FWD-Net

3.3.1.1. Description of collected data

FWD-Net currently covers surveillance on 18 diseases that are acquired by humans through the consumption of food or water, or contact with animals: anthrax, botulism, brucellosis, campylobacteriosis, cholera, cryptosporidiosis, echinococcosis, giardiasis, hepatitis A, leptospirosis, listeriosis, salmonellosis, shigellosis, toxoplasmosis, trichinellosis, typhoid/paratyphoid fever, verocytotoxin-producing *Escherichia coli* (VTEC)/Shiga toxin-producing *E. coli* (STEC) infection and yersiniosis. AMR data are collected as part

of the case-based datasets for salmonellosis and campylobacteriosis and, since 2013 data collection, as part of the molecular surveillance of *Salmonella* spp. and *Campylobacter* spp. isolates. The case-based dataset contains data from clinical treatment of patients and the results are therefore by default interpreted using clinical breakpoints for assessing treatment options. The isolate-based data are submitted by the National Public Health Reference Laboratories (NPHRLs) who do reference testing of isolates and can report the actual results of the antimicrobial susceptibility testing (AST) as minimum inhibitory concentration (MIC) or inhibition zone diameter.

Surveillance is conducted in accordance with Decision No 1082/2013/EU on serious cross-border threats to health, which in October 2013 repealed Decision No 2119/98/EC. The data collected by ECDC is published annually in the 'EU Summary Report (EFSA/ECDC, 2014) on AMR in Zoonotic and Indicator Bacteria obtained from Humans, Animals and Food thereof' which is produced in collaboration between ECDC and EFSA.

3.3.1.2. Strength of the system

Since 2012, major improvements have been made regarding the surveillance of AMR in *Salmonella* spp. and *Campylobacter* spp. in order to have comparable AMR data on zoonotic pathogens from humans between EU/EEA MSs and to have data comparable with those collected from animals and food. In June 2016, ECDC published an 'EU protocol for harmonised monitoring of AMR in human *Salmonella* and *Campylobacter* isolates' (ECDC, 2016a). The protocol targets the NPHRLs and covers, for example, EU surveillance objectives, the antimicrobial panels to test, test methods and reporting pathways.

The quality of the AMR data has improved significantly since then. It is now recommended that only results of AST performed at the NPHRLs are reported to ECDC and that all laboratories participate in the ECDC proficiency testing on AST for *Salmonella* spp. and *Campylobacter* spp. isolates. NPHRLs can report the results as measured values (MIC or zone diameter in mm) which means that the same interpretive criteria can be applied to the data collected according to the stipulated methods, whereas previously, a variety of national and international clinical criteria were used. The data can also be categorised according to epidemiological cut-off values (ECOFFs), which facilitates comparison with data collected from animals and food. The number of countries reporting data in this way has been increasing each year since it was introduced in the 2013 data collection. For 2015, approximately 60% of countries reported quantitative results.

In 2013–2015, AMR data were provided by the reporting countries for 16–19% of all laboratory-confirmed non-typhoidal salmonellosis cases and 12–18% of the laboratory-confirmed campylo-bacteriosis cases. Considering that about 95,000 salmonellosis cases and 230,000 campylobacteriosis cases were reported to ECDC in 2015, the AMR data provides a good overview of the AMR situation at EU level. The number of EU/EEA MSs reporting AMR data in 2013–2015 was 22–24 for *Salmonella* spp. and 15–19 for *Campylobacter* spp.

3.3.1.3. Impediments to comparing data

Many of the issues which were problematic for the comparison of data between countries and sectors in the first JIACRA report have been solved through the agreements made in the EU protocol and through the harmonisation efforts of EUCAST. Not all countries have been able to adhere to the protocol. Several countries only have access at the national level to the clinical interpretation of the test results, reported as clinically susceptible, intermediate or resistant. By combining the clinically intermediate and resistant categories, the resulting breakpoint is in most cases well aligned with the ECOFF. Regarding the antimicrobial panel tested and reported, in some cases, it reflects clinical practice rather than screening made for monitoring purposes and isolates which have results on many antimicrobials may indicate that the bacteria was resistant to the commonly used antimicrobials. The representativeness of data reported from the NPHRLs also varies between countries depending on the primary reason for referring isolates to the NPHRL (e.g. mainly in outbreak situations, focus on specific serotypes, only domestically acquired cases, when isolates are difficult to type, etc.), although a few countries refer all their *Salmonella* spp. isolates to the NPHRLs.

3.3.1.4. On-going actions to improve the system

ECDC plans to discuss and agree with the FWD-Net participants that AST results should be reported on isolates from a representative subset of all salmonellosis and campylobacteriosis cases captured in the national surveillance systems.

3.3.2. Surveillance of antimicrobial resistance in bacteria from humans through EARS-Net

3.3.2.1. Description of collected data

Monitoring of AMR in isolates from humans was conducted by MSs in accordance with Decision No 2119/98/EC (Official Journal of the European Communities, 1998)[1] setting up a network for the epidemiological surveillance and control of communicable diseases in the Community. For clinical isolates of bacteria from bloodstream infections (BSIs) and meningitis in humans, this is performed by the EARS-Net which is the largest publicly funded system of surveillance of AMR in Europe. EARS-Net is based on a network of representatives from the countries reporting routine clinical AST data from national AMR surveillance initiatives. Data are annually reported to ECDC and originate from approximately 900 laboratories serving more than 1,300 hospitals in Europe. Data are reported by EU/EEA MSs for the following eight pathogens/pathogenic species which are considered of public health importance: *E. coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Acinetobacter* spp., *Streptococcus pneumoniae*, *Staphylococcus aureus*, *Enterococcus faecalis* and *Enterococcus faecium*. Only invasive isolates (i.e. from blood and cerebrospinal fluid) are included in EARS-Net. The antimicrobial substance and pathogen combinations to be reported are defined in the EARS-Net reporting protocol. Data are reported as categorised AST results (susceptible, intermediate, resistant) on a single isolate basis. In addition, a number of countries provide quantitative results.

3.3.2.2. Strength of the system

A major strength of the EARS-Net surveillance is the use of a clear case definition for invasive isolates. EARS-Net data are exclusively based on invasive isolates from blood or cerebrospinal fluid. This restriction prevents some of the inconsistencies that otherwise arise from national differences in clinical case definitions, different sampling frames or heterogeneous health care. All 28 EU MSs, Norway and Iceland participate in EARS-Net. The majority of the participating countries have good national coverage, and many of the participating laboratories have reported data for several consecutive years, which enables accurate trend analyses.

3.3.2.3. Impediments to comparing data

Interpretation of the results of inter-country comparisons should be made with caution. A number of factors may introduce bias, resulting in over- as well as underestimation of resistance percentages. Some of the most important potential sources of bias are differences in the population coverage, sampling methods, laboratory routines and capacity. Moreover, case ascertainment of patients with BSIs is strongly linked to diagnostic habits and procedures, and the frequency by which blood cultures are taken. EARS-Net encourages the use of EUCAST clinical breakpoints; results based on other interpretive criteria used by the reporting countries are accepted for the analysis. Some countries report data from large national surveillance systems with a high national coverage, while other countries report data from a smaller subset of local laboratories and hospitals. In some countries, the population under surveillance is not constant and may change over the years.

3.3.2.4. On-going actions to improve the system

The quality of the antimicrobial susceptibility tests and procedures used by the laboratories are continuously measured through their participation in an annual external quality assessment (EQA) exercise offered to the participating laboratories. The EQA exercise is an important element of the surveillance system aiming to maintain and develop the ability of the laboratories to correctly determine susceptibility of bacterial isolates, and thereby ascertain the comparability of data reported to ECDC. Another on-going action of major importance for the quality of the surveillance system is the gradual implementation of EUCAST guidelines in the countries: at present, only 64% of the participating laboratories adhere to EUCAST guidelines. In addition, the EARS-Net reporting protocol is updated annually to reflect identified needs and continuously improve data quality.

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[1] As of 22 October 2013, Decision No 2119/98/EC was replaced by the Decision No 1082/2013/EU on serious cross-border threats to health.

3.4.   Monitoring antimicrobial resistance in bacteria from food-producing animals and food

Directive 2003/99/EC (Official Journal of the European Union, 2003) on the monitoring of zoonoses and zoonotic agents set out generic requirements for the monitoring and reporting of AMR in isolates of zoonotic *Salmonella* spp. and *Campylobacter* spp., as well as in selected other bacteria – in so far as they present a threat to public health – from food-producing animals and food in the EU MSs. Within the framework of AMR monitoring in food-producing animals and food, the occurrence of AMR is typically defined as the proportion of bacterial isolates tested for a given antimicrobial and found to present reduced susceptibility, i.e. to display 'microbiological resistance'. ECOFFs[2] are used as interpretative criteria of microbiological resistance.

3.4.1.   Description of collected data

3.4.1.1.   Data collected prior to 2014

In line with the general requirements of Directive 2003/99/EC, EFSA provided specific guidance on the monitoring and reporting of AMR in *Salmonella* spp. and *Campylobacter* spp. (EFSA, 2007) and in indicator *E. coli* and enterococci (EFSA, 2008). The monitoring of AMR in food-producing animals covered zoonotic agents, in the first instance *Salmonella* spp. and *Campylobacter* spp. on a mandatory basis, and indicator organisms in the commensal flora, such as *E. coli*, *Enterococcus faecium* and *Enterococcus faecalis*, on a voluntary basis. The monitoring of AMR in zoonotic organisms focused on the animal populations to which the consumer is most likely exposed through food derived thereof, such as domestic fowl (mainly broilers), pigs and cattle. The antimicrobials recommended for inclusion in the harmonised monitoring by EFSA consisted of a concise set of substances selected according to their relevance to human therapeutic use (e.g. critically important antimicrobials (CIAs) with highest priority for human medicine) and/or of epidemiological relevance. This set was used over the period 2007–2013 (Table 1).

3.4.1.2.   Data collected from 2014 onwards

The AMR monitoring in food-producing animals and food was revised by Commission Decision 2013/652/EU implementing Directive 2003/99/EC (Official Journal of the European Union, 2013), which set out monitoring priorities from a public health perspective and described those combinations of bacterial species, antimicrobial substances, food-producing animal populations and food products which should be monitored from 2014 onwards, including the frequency with which monitoring should be performed.

Since the implementation of the Commission Decision, the monitoring of AMR in zoonotic *Salmonella* spp. and *Campylobacter jejuni*, as well as in indicator *E. coli* from the major food-producing animal populations domestically-produced has become mandatory (Table 2). Indicator *E. coli* and *Campylobacter* spp. isolates derive from active monitoring programmes, based on representative random sampling of carcasses of healthy animals sampled at the slaughterhouse to collect caecal samples. For *Salmonella* spp. from broilers, laying hens and fattening turkeys, isolates are included which originate from salmonella national control plans, as well as isolates from carcases of broilers and fattening turkeys, sampled as part of process hygiene criteria. For *Salmonella* spp. from fattening pigs and bovine animals under 1 year of age, isolates are included originating from carcases of these animals, sampled as part of process hygiene criteria. The target number of organisms of each bacterial species which should be examined is 170 from each type of domestic animal (this is reduced to 85 organisms from poultry and pigs, if production is less than 100,000 tonnes per annum). From 2014 onwards, poultry/poultry meat will be monitored in 2014, 2016, 2018 and pigs and bovines under one year, pork and beef in 2015, 2017 and 2019. Within each MS, the various types of livestock and meat from those livestock should be monitored when production exceeds 10,000 tonnes slaughtered per year.

The antimicrobial substances included in the monitoring from 2014 onwards differ from those included in previous years and are shown in Table 1. The panel of antimicrobials tested includes those of particular current public health relevance as well as those of epidemiological relevance; ECOFFs

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[2] ECOFFs separate the naive, susceptible wild-type bacterial populations from isolates that have developed reduced susceptibility to a given antimicrobial agent (Kahlmeter et al., 2003). The ECOFFs may differ from breakpoints used for clinical purposes, which are defined against a background of clinically relevant data, including therapeutic indication, clinical response data, dosing schedules, pharmacokinetics and pharmacodynamics.

were used as the interpretative criteria of resistance (Kahlmeter et al., 2003). The harmonised panel of antimicrobials used, in particular for *Salmonella* spp. and *E. coli,* was broadened with the inclusion of substances, such as colistin and ceftazidime, that are either important for human health or provide clearer insight into the probable mechanisms of resistance to extended-spectrum cephalosporins.

Commission Implementing Decision 2013/652/EU (Official Journal of the European Union, 2013) stipulates that culture using selective media for cephalosporin-resistant *E. coli* should be performed. Caecal samples from broilers, fattening turkeys, fattening pigs and bovines under 1 year of age, as well as from broiler and turkey meat, pork and beef collected at retail should be examined for cefotaxime-resistant *E. coli* using selective media incorporating the 3rd-generation cephalosporin cefotaxime. This medium is selective for *E. coli* resistant to 3rd-generation cephalosporins and is expected to allow the growth of extended-spectrum beta-lactamase (ESBL), AmpC or carbapenemase enzyme producers which are resistant to cefotaxime at the microbiological cut-off. Three hundred caecal samples should be examined from each type of livestock.

All presumptive ESBL- or AmpC- or carbapenemase-producing *E. coli* isolates identified through the selective plating, as well as all those randomly selected isolates of *Salmonella* spp. and *E. coli*, recovered from non-selective media, that are resistant to cefotaxime or ceftazidime or meropenem, are further tested with a second panel of antimicrobial substances. This second panel of antimicrobials includes cefotaxime and ceftazidime with and without clavulanic acid (to investigate whether synergy is observed with clavulanate), as well as the antimicrobials cefoxitin, cefepime, temocillin, ertapenem, imipenem and meropenem. The second panel of antimicrobials is designed to enable phenotypic characterisation of ESBL, AmpC and carbapenem resistance.

Table 1: Harmonised set of antimicrobial substances used for the monitoring of resistance in zoonotic *Salmonella* spp. and *Campylobacter* spp., and indicator *E. coli* and enterococci isolates from food-producing animals and food over the periods 2007–2013[(a)] and from 2014 onwards[(b)]

Substances	*Salmonella* spp.		*C. coli*/*C. jejuni*		Indicator *E. coli*		Enterococci	
	–2013	2014–	–2013	2014–	–2013	2014–	–2013	2014–
Ampicillin	●	●			●	●	●	●
Azithromycin		●				●		
Cefotaxime	●	●			●	●		
Ceftazidime		●				●		
Chloramphenicol	●	●			●	●	●	●
Ciprofloxacin	●	●	●	●	●	●		●
Colistin		●				●		
Daptomycin								●
Erythromycin			●	●			●	●
Gentamicin	●	●	●	●	●	●	●	●
Linezolid							●	●
Meropenem		●				●		
Nalidixic acid	●	●	●	●	●	●		
Quinupristin/dalfopristin							●	●
Streptomycin				●			●	
Sulfonamides	●	●			●	●		
Teicoplanin								●
Tetracyclines	●	●	●	●	●	●	●	●
Tigecycline		●				●		●
Trimethoprim	●	●			●	●		
Vancomycin							●	●

–2013: up to and including 2013; 2014–: from 2014 onwards.
(a): EFSA (2007, 2008).
(b): Commission Decision 2013/652/EU (Official Journal of the European Union, 2013).

Table 2: Organisms included in AMR monitoring in 2014 and subsequent years, as set out in Commission Implementing Decision 2013/652/EU

Animal population	*Salmonella* spp.	*C. jejuni*	*C. coli*	*E. coli*	*E. faecalis/faecium*
Broilers	M, NCP, PHC	M, CSS	V	M, CSS	V
Laying hens	M, NCP	–	–	–	–
Fattening turkeys	M, NCP, PHC	M, CSS	V	M, CSS	V
Bovines < 1 year	M, PHC	–	–	M, CSS	V
Fattening pigs	M, PHC	–	–	M, CSS	V

CSS: caecal samples from healthy animals at slaughter; M: mandatory monitoring; NCP: *Salmonella* national control plans; PHC: process hygiene criteria; V: voluntary monitoring.

3.4.2. Strength of the system

The monitoring of AMR in food-producing animals under Commission Implementing Decision 2013/652/EU (Official Journal of the European Union, 2013) covers the main food-producing animal species and where appropriate, includes different production sectors (for example, broilers and laying hens). Randomised, representative sampling is no longer stratified at the level of the different animal species (e.g. *Gallus gallus*, cattle, pigs) but rather is performed at the level of the major food-producing animal production categories, such as broilers, laying hens, fattening pigs, fattening turkeys and bovines under 1 year of age.

The effects of consumption of antimicrobials in a given country and animal species, on the occurrence of resistance, can be studied more easily in indicator organisms than in foodborne pathogens, such as *Salmonella* spp., because all food-producing animals generally carry these indicator bacteria. An important feature from the perspective of the second JIACRA report is that monitoring resistance in indicator commensal *E. coli* has become mandatory and that sampling of indicator bacteria should be representative of the domestically produced population studied, in accordance with the provisions of the Decision and the detailed technical specifications issued by EFSA (EFSA, 2012, 2014). The isolates subjected to susceptibility testing have typically been derived from active monitoring programmes in healthy animals, ensuring representativeness of resistance data, especially in the case of indicator bacteria and *Campylobacter* spp., whereas AMR data from susceptibility testing of *Salmonella* spp. have remained more dependent on the prevalence and the serovar distribution of the bacteria in the different animal populations.

The Commission Decision 2013/652/EU (Official Journal of the European Union, 2013) also ensures that all reporting countries submit data for a common core set of antimicrobials and bacteria set out in Table 2; data for these combinations should therefore be comprehensive. The collection and reporting of data is now performed at the isolate level, which enables in depth analyses to be conducted, in particular on the occurrence of multidrug resistance. The analysis of the results at individual isolate level also allows investigation of possible associations between the occurrence of isolates which are fully-susceptible to the panel of antimicrobials tested and the consumption of antimicrobials.

3.4.3. Impediments to comparing data

Regarding the AMR data obtained before 2014, EFSA provided detailed specifications on minimum requirements (EFSA, 2007, 2008) for the harmonised monitoring of AMR in food-producing animals so that comparable data was obtained across the EU/EEA MSs and Switzerland. Nevertheless, up to 2013, monitoring of resistance in indicator *E. coli* and enterococci was performed on a voluntary basis and limited data were reported by a number of reporting countries to EFSA.

3.4.4. On-going actions to improve the system

An external quality assurance system, based on regular training and yearly proficiency tests, is included in the AMR monitoring programmes. This will detect potential differences between the laboratories performing susceptibility tests relating to methods or interpretative criteria and is coordinated by the National Reference Laboratories on AMR within each reporting country and the EU Reference Laboratory on Antimicrobial Resistance.

The EC has also carried out audits in, up to now, 14 MSs to check the actual implementation of the AMR monitoring programmes laid down by the new EU legislation. The system therefore ensures that

there is harmonisation of resistance monitoring in food-producing animals and comparability of the AMR data recorded in the respective EU MSs.

4. Methodological considerations and included data

4.1. Analytical approaches

The availability and characteristics of data at the European level on AMC and AMR to selected antimicrobials in both humans and food-producing animals and food derived thereof have been summarised in the earlier sections. Obtained from the monitoring systems in place in the reporting countries, four sets of data are available, corresponding to AMC and AMR in both human and animal populations. These four sets of data and the potential relationships between them are illustrated in Figure 1.

The analytical approach followed in this report addressed primarily the relationship between consumption and resistance within the animal and human populations – as illustrated by the vertical arrows in Figure 1. The approach also considered potential additional associations between equivalent data from the two populations: AMR in humans vs AMR in animals, and AMC in humans vs AMC in animals – as illustrated by the horizontal arrows in Figure 1. In fact, any positive association between resistance data in humans and in animals might reflect the transfer of resistant bacteria between human and animal populations and/or some similarities in the consumption of antimicrobials among human and animal populations. Assessing the existence of these horizontal links will provide relevant information for assessing a potential relationship between AMC in animals and AMR in humans – as illustrated by the diagonal arrow in Figure 1. The existence of those potential relationships was investigated through a series of univariate analyses addressing selected antimicrobial class/bacterial organism combinations of interest. The relationship between AMC in humans and AMR in food-producing animals was not addressed in this report.

Secondarily, the analytical approach followed in this report included multivariate analyses addressing the selected antimicrobial class/bacterial organism combinations of interest to assess simultaneously relationships between AMR in bacteria from humans and AMC in both human and animal populations as well as AMR in bacteria in animals, while still accounting for the characteristics of the data analysed, in particular, the relatively small number of observations – number of countries – involved in the ecological analysis and multicollinearity among dependent variables.
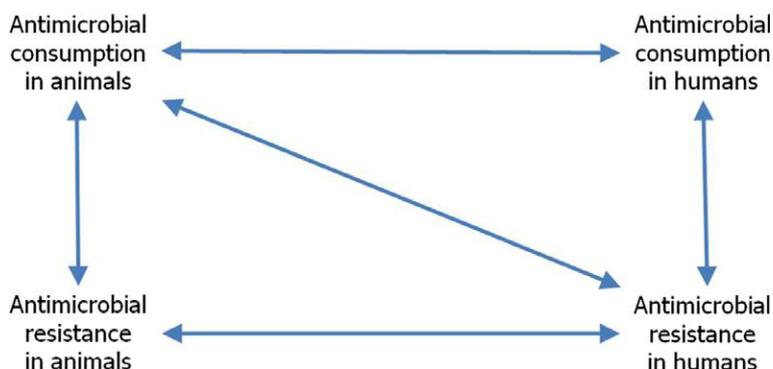
Note: The relationship between AMC in humans and AMR in food-producing animals was not addressed in this report.

Figure 1: Available sets of data related to AMC and AMR in humans and food-producing animals in the reporting countries and the possible relationships investigated in this report

4.2. Rationale for selecting antimicrobial/organism combinations

In the current report, only data on AMR obtained in domestically produced animals have been used in the analyses, as available data on AMR in bacteria recovered from meat (broiler meat, pork and beef) as well as related information on the meat origin - domestically produced or imported – were considered insufficient (i.e. there were too few reporting MSs) for a meaningful investigation of associations between the consumption of antimicrobials in animals and the occurrence of AMR in

certain bacteria present on meat. Applying a similar principle, the analyses presented did not attempt to evaluate AMC and AMR for all available combinations of antimicrobials and bacterial organisms, but conversely, were carried out only for selected antimicrobial classes and organisms, which are considered to be particularly important.

The WHO list of critically important antimicrobials (WHO, 2017) and the EU Antimicrobial Advice ad hoc Expert Group (AMEG) list (EMA/AMEG, 2014, 2016) were taken into account when selecting the combinations of antimicrobials and bacterial organisms for detailed analysis. In particular, fluoroquinolones, polymyxins and 3rd- or 4th-generation cephalosporins have been considered as three of the classes of antimicrobial agents most urgently requiring management of the risks from AMR. The 3rd- and 4th-generation cephalosporins and fluoroquinolones have much more recently been introduced into veterinary medicine than old compounds, such as the tetracyclines. Therefore, in most of the reporting countries, resistance to tetracyclines is relatively common in many bacteria from animals. This differs in many (but not all) cases from the situation for fluoroquinolones and 3rd- and 4th-generation cephalosporins.

In order for a bacterium with an animal reservoir to cause infection in humans via ingestion of meat, the bacterium needs to survive the meat production chain and also to be infectious to humans. *Salmonella* spp. and *Campylobacter* spp. are well-recognised causes of foodborne zoonoses and, although infections in humans may arise from imported food or be related to travel, it is considered important to include these bacteria. *Salmonella* spp., in particular, can show extensive resistance, thus compromising treatment options in both humans and animals when treatment is considered necessary.

In the case of *E. coli*, some types which can infect humans, particularly pathogenic VTEC types such as *E. coli* O157:H7, have their primary reservoir in food-producing animals. Moreover, several studies recognise that a proportion of *E. coli* involved in human infections may originate from food-producing animals, including AMR isolates (Lazarus et al., 2014; Manges and Johnson, 2012). The proportion of *E. coli* infections in humans, other than VTEC, with a zoonotic origin is unknown, and further studies are needed to quantify the transfer of such organisms from food-producing animals to humans. Most analyses on AMR *E. coli* from cases of infection in humans have been performed on isolates from BSIs. Such isolates probably possess a variety of virulence genes and virulence mechanisms not found in commensal *E. coli* from food-producing animals.

The combinations of classes of antimicrobial and bacterial organisms finally prioritised for analysis are shown together with main concepts of rationale for selection in Table 3.

Table 3: Combinations of antimicrobial classes and bacterial organisms selected for analysis and rationale for the selection

Antimicrobial class	WHO and AMEG classification	*Campylobacter* spp.	*Salmonella* spp.	*E. coli*	*Klebsiella* spp.
3rd- and 4th-generation cephalosporins	• Highest priority CIAs for WHO • Category 2 of AMEG list		• Resistance to 3rd- or 4th-generation cephalosporins in *Salmonella* spp. and in *E. coli* is addressed as this antimicrobial class constitutes one of the first-line therapies for invasive Gram-negative bacterial infections in humans in many EU MSs		
Fluoroquinolones and other quinolones	• Highest priority CIAs for WHO • Fluoroquinolones Category 2 of AMEG list, other quinolones Category 1.	• Fluoroquinolones and macrolides are used to treat infections with *Campylobacter* spp. in humans when treatment is considered necessary by the clinician	• Resistance to fluoroquinolones in *Salmonella* spp. and in *E. coli* is addressed because this antimicrobial class constitutes one of the first-line therapies for invasive Gram-negative bacterial infections in humans in many EU MSs		
Macrolides	• Highest priority CIAs for WHO • Category 2 of AMEG list				
Tetracyclines	• HIAs for WHO • Category 1 of the AMEG list	• Broad-spectrum antimicrobial class widely used in animals for many years • Resistance to tetracyclines and to other antimicrobials, which is common, may play in co-selection through the genetic linkage of resistance genes • There are different patterns of use in humans and animals in the EU			
Polymyxins	• Highest priority CIAs for WHO • Category 2 of AMEG list		• The notable increase in resistance (mainly chromosomally-mediated) to the polymyxin antimicrobial colistin in Enterobacteriaceae in several countries in southern Europe since 2010 (ECDC, 2015; EMA/AMEG, 2016) is of concern, because of the rapidly increasing use of colistin in EU/EEA hospitals • High consumption of colistin has been reported in food animals in some countries (EMA/AMEG, 2016); it is increasing in the hospital sector, mainly in intensive care units (and to a lesser extent in the community sector for specific patient populations, i.e. patients with cystic fibrosis)		

Antimicrobial class	WHO and AMEG classification	*Campylobacter* spp.	*Salmonella* spp.	*E. coli*	*Klebsiella* spp.
Carbapenems [a]	• Highest priority CIAs for WHO • Category 3 of AMEG list		• Resistance to carbapenems is emerging in several bacterial species capable of causing serious, invasive infections in humans • Although this class of antimicrobials is not authorised for use in animals in the EU, carbapenem resistance in bacteria from animals has been detected sporadically • Carbapenems are antimicrobials of major clinical significance for which the epidemiology of resistance seems as yet not to include a significant animal reservoir of resistant organisms (EFSA BIOHAZ Panel, 2013), though recent reports from Germany have indicated that the situation might not be static (Borowiak et al., 2017)		

AMEG: Antimicrobial Advice ad hoc Expert Group; CIA: critically important antimicrobial; HIA: highly important antimicrobial; WHO: World Health Organization.

A grey cell means that the corresponding combination was not addressed in the analysis.

(a): Not authorised for use in animals in the EU.

4.3. Overall consumption of antimicrobials in humans and food-producing animals

National representative consumption data on the quantity of antimicrobials for systemic use in humans (ATC group J01) and based on sales or reimbursement data sources were reported by ESAC-Net as numbers of DDD per 1,000 inhabitants per day. Figures of consumption in 2013, 2014 and 2015 were retrieved from the TESSy database hosted by ECDC in January 2017. To facilitate the comparison between AMC in humans and in animals, these data were subsequently converted into mass of active substance per antimicrobial class and country (expressed in tonnes). Detailed information on the conversion methodology used can be found in Appendix B.2. Where available, data on consumption in the hospital sector and in the community were aggregated to provide total consumption. For those countries reporting on community consumption only, this figure was used as a surrogate for the total consumption.

Figures for consumption of antimicrobials in food-producing animals, expressed in tonnes, for the years 2013 and 2014 were retrieved from the ESVAC database hosted by EMA on 1 December 2016. The addressed antimicrobials belonged to the following ATCvet groups: QA07A, QG01A, QG01B, QG51A, QJ01, QJ51 and QP51A.

Data on the human populations covered by the surveillance of AMC were obtained from the ESAC-Net TESSy-database in January 2017 and were based on either Eurostat or national population data sources. Data on the average weights of different age groups (EFSA, 2012) were used together with Eurostat data on the population in EU-27 in 2012 by 1-year age classes to calculate a human EU population and age class weighted average body weight of 62.5 kg (see Appendix B.3). This body weight was used to calculate the estimated biomass of the population under ESAC-Net surveillance.

Data on the biomass of food-producing animals expressed in PCU for the period 2013–2014 were obtained from the ESVAC-database hosted by EMA on 1 December 2016. In the following, the term 'milligrams per kilogram of estimated biomass' will be used as a synonym of 'milligrams per human EU population- and age class-weighted biomass' and 'milligrams per PCU'.

4.4. Technically derived estimates of the sales of veterinary antimicrobials for pigs and poultry

In the absence of AMC data specifically monitored in pigs and poultry in most of the EU/EEA MSs, the sales of the antimicrobial classes/subclasses included in the analysis at the animal species level were estimated by use of a structured approach. Sales data from the ESVAC database for the years 2013–2015 were used to establish estimates of sales in pigs and poultry for antimicrobial veterinary medicinal products (VMP) belonging to ATCvet groups QA07AA and QJ01 corresponding to 3rd- and 4th-generation cephalosporins, fluoroquinolones, other quinolones, polymyxins, macrolides and tetracyclines. Data on sales in 2013 and 2014 were retrieved from the ESVAC database hosted by EMA on 21 November 2016, and included updates made to the historical data and for 2015 values were retrieved on 1 February 2017.

For each of the antimicrobial VMP presentations included in the analysis, information on authorised target food-producing species was obtained from the Summary of Product Characteristics (SPC) of each country. Subsequently, the total annual sales (weight of active ingredient) of each VMP presentation were distributed between the authorised target food-producing species according to its biomass – i.e. the population correction unit (PCU) ratio in the corresponding country. The biomass ratio for pigs and poultry is defined as the fraction of the biomass (PCU) of these species of the total animal biomass (PCU) in the respective country. For some VMPs, the SPC data indicated poultry as target species and in order to have harmonised data across MSs, sales were estimated for poultry (and thus AMR data for turkey and broilers were aggregated for the analyses of the second JIACRA report). The AMR data used for the analyses in the current report cover bovine animals under 1 year but since cattle in general is typically given as target species in SPC's, sales for bovines under 1 year of age could not be estimated by the approach used.

The sales (weight of active substance) attributed to pigs and poultry were subsequently used to calculate the indicator used to express a proxy for the exposure to antimicrobials – i.e. number of defined daily doses animals (DDDvet[3]) per kilogram of animal biomass per species (DDDvet/kg biomass) per year and country. The DDDvet system, established by EMA, provides standardised fixed units of measurement for the reporting of data on consumption by species and take into account

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[3] Available online: http://www.ema.europa.eu/ema/index.jsp?curl=pages/regulation/general/general_content_001493.jsp&mid=WC0b01ac0580a2fcf5

differences in dosing between species, antimicrobials and administration routes/formulations. The principles for assignment of DDDvet (EMA/ESVAC, 2015) are harmonised with the principles for assignment of DDDs in human medicine to the greatest extent possible. Similar to the DDD established for human medicinal products, DDDvet is a technical unit of measurement solely intended for drug consumption studies and therefore cannot be assumed to represent the real daily doses applied.

It should be emphasised that the estimates obtained on sales for pigs and poultry by use of this methodology are purely technically derived estimates; consequently, the numbers of DDDvet per kilogram of animal biomass per year and country should not be interpreted as the actual exposure to antimicrobials in pigs and poultry in the ESVAC participating countries (see also Appendix B.4).

4.5. Data on antimicrobial resistance in bacterial isolates from food-producing animals

4.5.1. Summary indicator of resistance in bacterial isolates from animals

For the purpose of comparing AMC and AMR data, a summary indicator of microbiological resistance (SIMR) at MS level was calculated as the weighted mean of the proportion of AMR in cattle, domestic fowl and pigs for the year 2013, and as the weighted mean of the proportion of AMR in broilers, turkeys, pigs and calves (bovine under 1 year of age) considering AMR data assessed in 2014 and 2015. The PCU values of the three/four (or two when considering *Campylobacter* spp. data) animal categories in the MSs were used as weighting factors. An additional SIMR in bacteria from poultry was also constructed by addressing data on both broilers and turkeys for the year 2014. For the MSs for which AMR in turkeys were not available because of a small size of the turkey production sector, the SIMR in poultry equaled the occurrence of AMR assessed in broilers. These differences reflect changes in the EU legislation on AMR monitoring in food-producing animals implemented in 2014. SIMR were compared to corresponding AMC data in food-producing animals.

4.5.2. Data for assessing the impact of co-selection

In the animal sector, the reporting of AMR data at individual isolate level allows characterisation of phenotypic profiles of resistance to the harmonised panel of antimicrobial substances tested. This also enables analysis of complete susceptibility, multi-drug resistance (to three or more antimicrobial classes) (MDR) and co-resistance patterns to highest priority CIAs.

In the human sector, data on invasive *E. coli* from humans focus on antimicrobials used for human treatment and does therefore not include all antimicrobials present on the animal panel. In addition, quantitative MIC data would be needed to interpret the results with ECOFFs instead of the clinical breakpoints which are generally reported. In 2015, MIC data were available for 23% of the invasive *E. coli* isolates and the situation was similar for 2013 and 2014. These data were not validated and often MIC ranges were truncated. Of 20 countries providing any MIC data, most only reported this for a small proportion of the isolates, whereas three countries provided MIC for almost 100% of the *E. coli* isolates. For most of the remaining countries, providing MIC data, the MIC subset was regarded as not representative of the data from the individual countries. For these reasons, the co-selection analysis was not considered appropriate for application to the data on invasive *E. coli* from humans.

4.5.2.1. Accounting for multiple resistance traits

Based on a method derived from that proposed by Søgaard (1989) and reviewed by Monnet et al. (2001), an attempt was made to empirically account for the co-selection phenomenon when comparing AMC and AMR in food-producing animals. The analysis assumes that an observed occurrence of AMR is the result of the simultaneous actions of several antimicrobials on a given bacterial population because of the MDR traits of the population, which may lead to co-selection. Various fractions of the consumption of these antimicrobials should be taken into account while modelling the relationship between consumption and AMR (see Appendix B.5).

4.5.2.2. Addressing complete susceptibility

In the animal sector, the reporting of AMR data at the individual isolate level allowed characterisation of phenotypic profiles of resistance to the harmonised panel of antimicrobial substances tested. A completely susceptible indicator *E. coli* isolate is one defined as non-resistant to all of the antimicrobial substances included in the harmonised set of substances tested. Associations were investigated between the occurrence of complete susceptibility and total AMC in food-producing animals.

4.6.   Data on antimicrobial resistance in bacterial isolates from humans

4.6.1.   *Salmonella* spp. and *Campylobacter* spp.

The method of testing for antimicrobial susceptibility and the selection of the isolates to be tested varied between countries. The methods and interpretive criteria used for AST of *Salmonella* spp. and *Campylobacter* spp. isolates from humans can be found in the corresponding EFSA/ECDC reports (EFSA/ECDC, 2015, 2016, 2017). Quantitative data were interpreted by ECDC based on the EUCAST ECOFF values, where available. Where ECOFFs do not exist, EUCAST or Clinical and Laboratory Standards Institute (CLSI) clinical breakpoints were applied. For the qualitative SIR data, intermediate and resistant results were combined into a non-susceptible category. Alignment of the susceptible category with the 'wild-type' category based on ECOFFs and of the non-susceptible category with the ECOFF-based 'non-wild-type' category provides better comparability and more straightforward interpretation of the resistance data for most antimicrobial agents included. When analysed in this way, there is generally close concordance ($\pm$ 1 dilution) across categories for the antimicrobials included for *Salmonella* spp. and *Campylobacter* spp. (Figures 2 and 3).
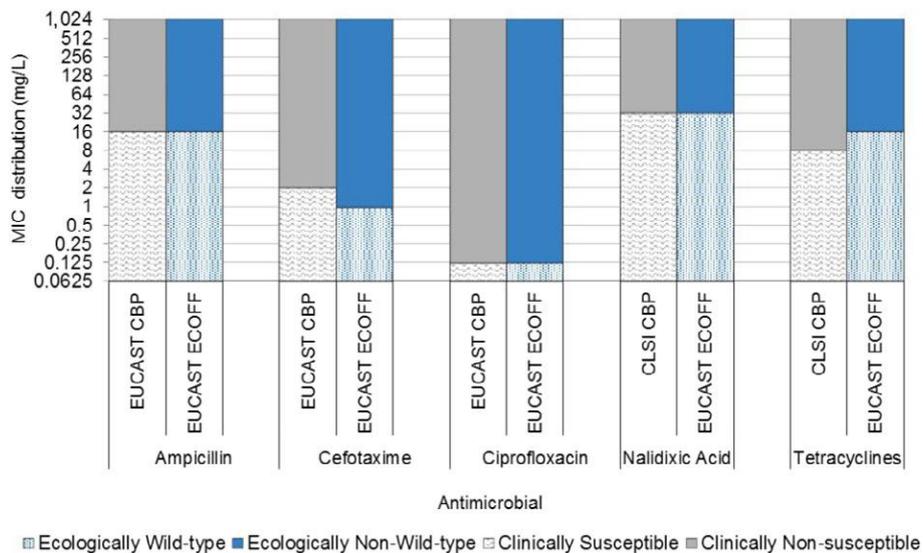
Figure 2: Comparison of clinical breakpoints for resistance (intermediate and resistant categories combined) and epidemiological cut-off values used to interpret MIC data reported for *Salmonella* spp. from humans and food-producing animals
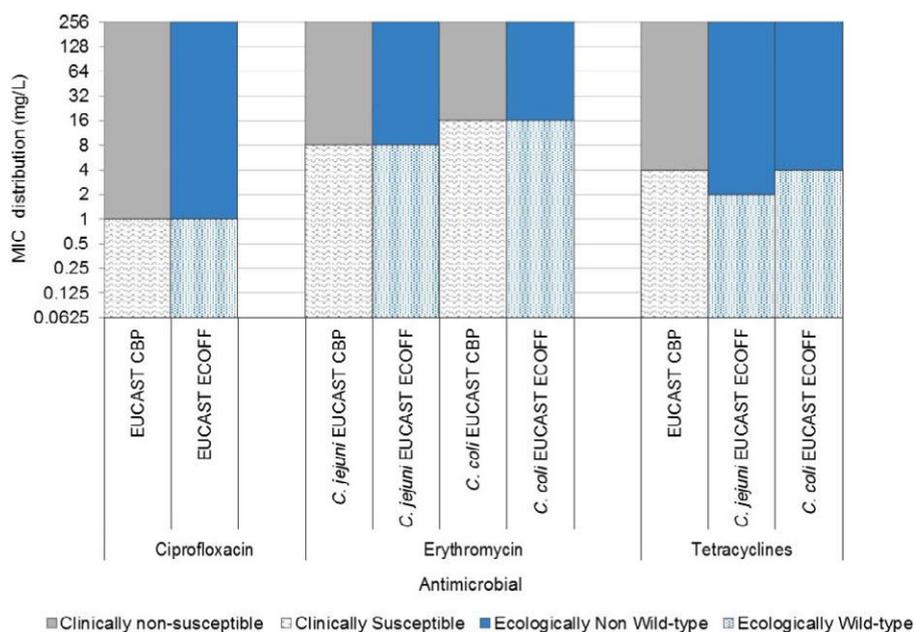
Figure 3: Comparison of clinical breakpoints for resistance (intermediate and resistant categories combined) and epidemiological cut-off values used to interpret MIC data reported for *Campylobacter* spp. from humans and food-producing animals

Low-level fluoroquinolone resistance in *Salmonella* spp. may be difficult to detect with ciprofloxacin using disk diffusion and nalidixic acid has therefore been used as a marker for low-level fluoroquinolone resistance. Plasmid-mediated fluoroquinolone resistance is often not detected with nalidixic acid. Since 2014, EUCAST therefore recommend to use pefloxacin for screening of low-level fluoroquinolone resistance in *Salmonella* spp. with disk diffusion (EUCAST, 2014). Nalidixic acid is still better at detecting resistance in isolates having the *aac(6′)-Ib-cr* gene as the only resistance determinant (Skov et al., 2015). While both ciprofloxacin and nalidixic acid have been part of the antimicrobial panel for *Salmonella* spp. in humans for years, countries have implemented the switch to pefloxacin at different times during the study period. Because of this, the AMR results of ciprofloxacin, nalidixic acid and pefloxacin were combined for *Salmonella* spp., i.e. an isolate was considered resistant to fluoroquinolones if exhibiting resistance to any of the three antimicrobials listed above.

Travel-associated infections acquired outside of the reporting country were excluded from the dataset in order not to bias the proportion of resistant isolates. As several countries had not provided any information on travel (or non-travel) of their cases, cases with unknown travel status were included in addition to domestically acquired cases.

4.6.2. Invasive *E. coli* and *K. pneumoniae*

The method of testing for antimicrobial susceptibility in invasive *E. coli* and *K. pneumoniae* in humans and the selection of the isolates to be tested varied between countries. A large majority of the Member States use EUCAST clinical breakpoints, while a few laboratories still use Clinical and Laboratory Standards Institute (CLSI) clinical breakpoints. This could result in discrepancies between data when the resistance mechanisms result in MICs close to the breakpoints. For more information, the reader should refer to the EARS-Net reports (ECDC, 2014a, 2015, 2017a).

In order to compare AMR between clinical isolates of invasive *E. coli* from humans and commensal *E. coli* from food-producing animals, clinically intermediate resistant and clinically resistant results were combined into a non-susceptible category in the *E. coli* data from humans. This approach did not provide as good alignment with the categories based on ECOFFs as it did for *Salmonella* spp. and *Campylobacter* spp. and there was a difference of one to four dilution steps, depending on antimicrobial, between the non-susceptible clinical levels and the non-wild type (microbiologically resistant) based on ECOFFs (Figure 4). For consistency, clinically intermediate resistant and clinically resistant results for *K. pneumoniae* was also merged into a non-susceptible category, even though no comparisons were made with data from food-producing animals.
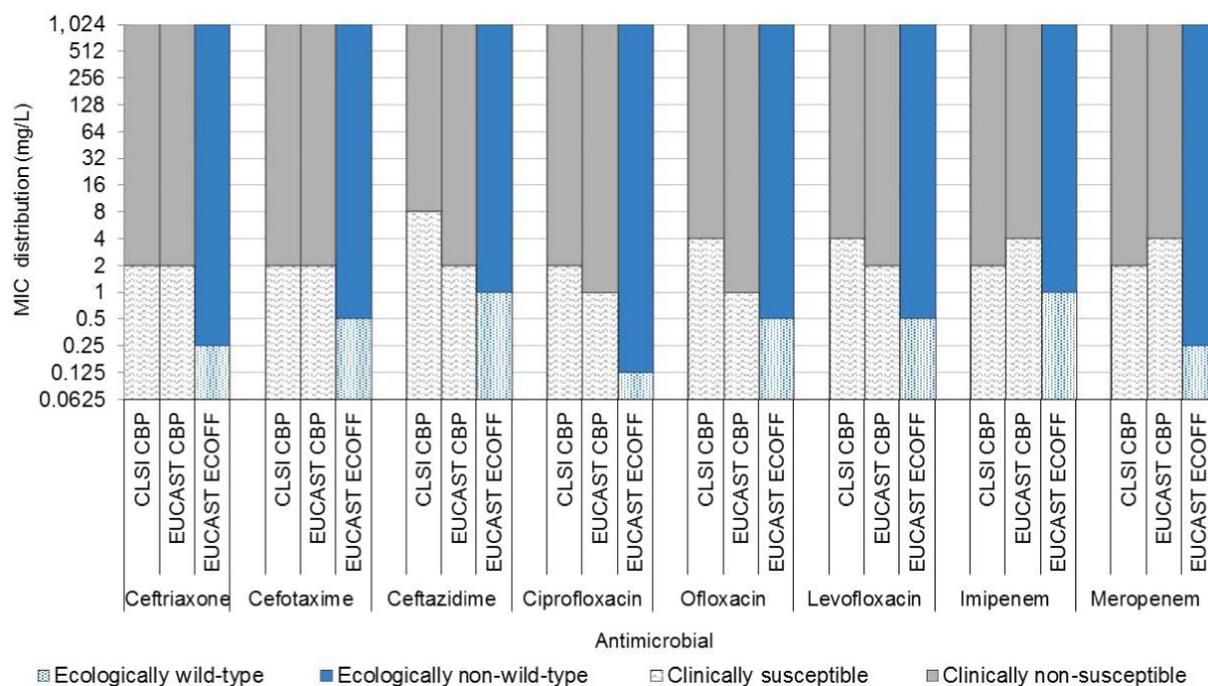
Figure 4: Comparison of clinical breakpoints for resistance (intermediate and resistant categories combined) used for invasive *E. coli* from humans and epidemiological cut-off values used to interpret MIC data reported for indicator *E. coli* from food-producing animals

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Comparison of resistance between bacteria from humans and food-producing animals

As described in previous Sections (3.4, 4.6.1 and 4.6.2), resistance in bacteria from food-producing animals and part of the data on *Salmonella* spp. and *Campylobacter* spp. from humans were assessed using epidemiological cut-off values to represent 'microbiological' resistance. Resistance in invasive bacteria form humans and part of the data on *Salmonella* spp. and *Campylobacter* spp. from humans were reported using clinical breakpoints and intermediate and clinical resistance was then combined to align with the 'microbiological' resistance. This procedure results in perfect or very close concordance (± one dilution) across categories for the antimicrobials included for *Salmonella* spp. and *Campylobacter* spp. while for *E. coli*, differences of up to four dilutions still prevail. This means that indicator *E. coli* isolates from animals would more frequently be classified as resistant compared to *E. coli* isolated in humans. While this report does not directly compare the levels of occurrence of resistance between animals and human isolates, this difference may still have an impact on the results of the logistic regressions.

4.7.   Statistical methodologies

4.7.1.   Spearman's rank correlation test

To assess whether there was an association between AMC (expressed in milligrams per kilogram of estimated biomass) of antimicrobials in animals and in humans at the EU level, a Spearman's rank correlation test was used. Spearman's rank correlation coefficient is a non-parametric measure used to assess the degree of statistical association between two variables and the test does not depend on any assumptions about the distribution of the data. The Spearman's rank correlation coefficient is identified by rho (ρ) and varies from -1 (complete negative correlation) to 1 (complete positive correlation).

4.7.2.   Logistic regression

To assess the potential associations between (1) AMC in humans and occurrence of resistance in bacterial isolates from humans, (2) AMC in food-producing animals and occurrence of resistance in isolates from food-producing animals, (3) occurrence of resistance in isolates from food-producing animals and occurrence of resistance in isolates from humans, and (4) AMC in food-producing animals and occurrence of resistance in isolates from humans, logistic regression models were fitted for each of the studied associations for the years 2013, 2014 and 2015 (or 2014–2015), separately. Logistic

regressions were modelled only for those associations where five or more countries reported information on both the outcome of interest and predictor and where the total number of isolates tested within each country was equal to 10 or more. Logistic models were performed using the LOGISTIC procedure of SAS software (Institute SAS, 1999).

4.7.2.1. Measure of association

The odds ratio (OR) was used to assess the strength of association between the predictor and the outcome of interest and 95% confidence intervals (CIs) for ORs were calculated. The OR represents the odds that the outcome of interest will occur given a particular exposure, compared to the odds of the outcome of interest will occur in the absence of that exposure. Statistical significance was identified with the p-value. The p-value, or calculated probability, is the probability of finding significance in the studied association. The p-value is also described in terms of the null hypothesis H_0. The null hypothesis in this study states that no statistically-significant association was present between the predictor and the outcome of interest. The level of significance was set at 0.05. A p-value of 0.05 or less meant that the null hypothesis was rejected and that a statistically-significant association was found between the predictor and the outcome of interest.

4.7.2.2. Model

Logistic regressions were performed estimating logit models with grouped data (each country being a group) and accounting for small sample sizes (number of countries involved) and possible over-dispersion, which may arise when estimating a logit model with grouped data – deviance and Pearson chi-square are large, relative to the degrees of freedom. The logistic regression deals naturally with the binomial nature of the event of interest (reduced sensitivity vs naive sensitivity in animals; clinical resistance vs sensitivity in humans). To account for possible over-dispersion, the over-dispersion correction proposed by Williams (1982) and offered by the LOGISTIC procedure of SAS software was used. CIs for logit regression coefficients were computed by profile likelihood (PL) which produces more robust approximations, especially in smaller samples. The likelihood ratio test was used to assess the goodness-of-fit of the models, which investigates how well the modelled data represents the observed data.

4.7.2.3. Outputs

Next to the tabulated outputs, graphical outputs are plotted in the report. The graphs and tables present all the data that were included in the study. The graphs show the fitted logistic curve (with prediction bands), which represents a plot of the estimated probability of AMR vs the AMC, as single predictor. The graphs also reveal country distributions and differences that are not readily apparent in tabular output. Where deemed necessary, the scale used in the graphs is adapted according to the range of probabilities of resistance which was observed, in order to best show the distribution of data points. Data outliers – outlying countries on the graphs – were identified by visual inspection of the graphs and omitted in a subsequent sensitivity analysis, when considered appropriate. The results of the sensitivity analyses, when found relevant, were commented in the text. When analysing the potential relationships between human AMC and AMR and within MVA, a sensitivity analysis or interpolation of missing hospital sector data was performed to address this potential drawback.

4.7.3. Partial Least Squares Path Modeling

To further assess simultaneously potential relationships between the resistance of bacteria from humans to antimicrobials and AMC in humans (in the community and at the hospital), AMC in food-producing animals (pigs and poultry) and resistance in bacteria from food-producing animals (pigs and poultry), multivariate analyses were performed by using Partial Least Squares Path Modeling (PLS-PM). PLS-PM was selected, as a convenient tool to investigate multiple relationships between blocks of variables (represented through latent variables as a mean of summarising measured variables into fewer factors) without requiring assumptions on data distributions.

4.7.3.1. Data addressed in the multivariate analyses

Multivariate analyses were based on data reported for 2014 and 2015. Data on AMR in isolates from pigs and poultry were recorded in 2015 and 2014, respectively. Data on AMR in isolates from humans were calculated by pooling the corresponding data collected in 2014 and 2015, and AMC in humans was calculated as the average of 2014 and 2015 data. For countries that did not report AMC

data at the hospital, hospital consumption was estimated by making use of other countries' partition between hospital and community consumption (regression procedure). For those countries where data were not available or validated when performing the analyses to estimate AMC in pigs in 2015, 2014 estimates were used as a proxy for 2015. All data were standardised (i.e. mean = 0 and variance = 1) prior to inclusion in the model.

4.7.3.2. Outcomes

The typical outcomes available of PLS-PM are presented in Table 4 and illustrated on Figure 5.

Table 4: Outcomes of PLS-PM models used in the multivariate analyses

Outcomes	Characteristics
R^2	Indicates the amount of variance in the dependent variable explained by the independent latent variables. Its value is usually considered high when it is greater than 0.50 or 0.60, depending on the authors
Weights	Usually placed next to the corresponding arrow (Figure 5), it represents the relative contribution of an indicator to the definition of the corresponding latent variable, varying from 0 to < 1, the greater the value, the 'stronger' the model
Path coefficients (β)	Usually placed next to the corresponding arrow, they are coefficients of the paths between latent variables, which vary between −1 and +1, and are standardised. The closer to |1| the coefficient, the stronger the path
Effects – **Direct effects** – **Indirect effects**	Corresponds to the effect of one latent variable on another one. It corresponds to the path coefficient when the effect is direct, but is termed an indirect effect when a latent variable mediates this effect indirectly, such as the indirect effect of AMC by animals on resistance in human isolates, mediated by resistance in animals
Redundancy	Reflects the ability of independent latent variables to explain variation in the dependent latent variable. The larger the value, the greater the ability of the model to predict AMR in bacteria from humans

4.7.3.3. Full initial model

The full initial model computed is presented with related outcomes (Figure 5), according to the usual representation of PLS-PM. Indicators, also called 'manifest variables', are presented in rectangles; they correspond to measured data on AMR and AMC. The variables displayed in ovals are 'latent variables', which were obtained from 'manifest variables'. Models were formative ones since latent constructs were formed by its indicators, as shown by arrows going from rectangles to ovals on Figure 5.
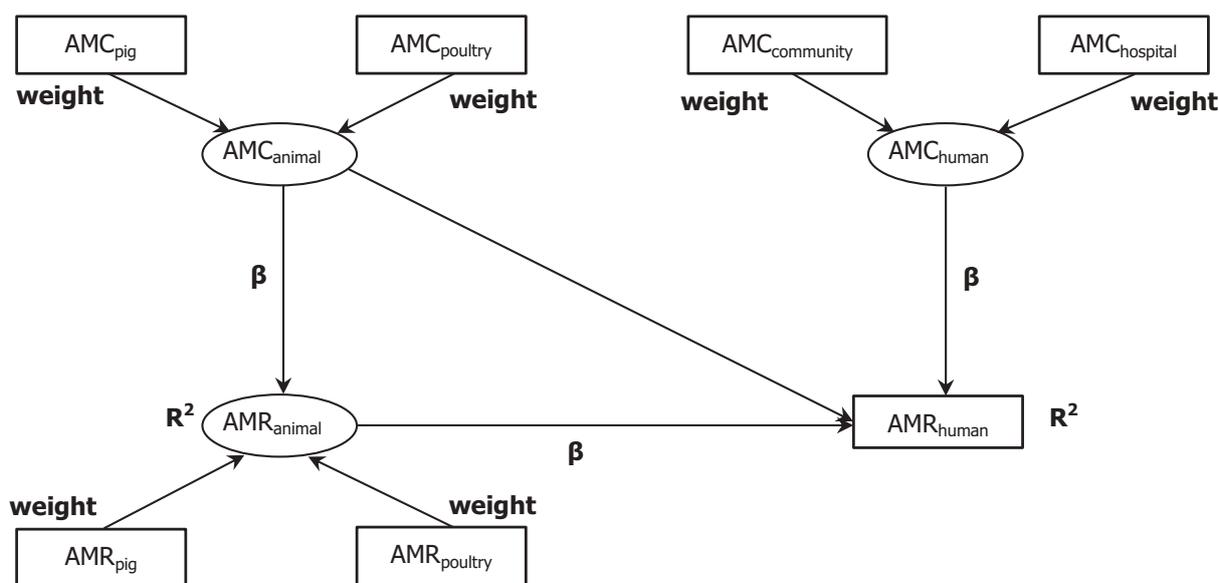
Figure 5: Diagram showing the initial model considered to assess the potential relationships between antimicrobial resistance in bacteria from humans (AMR$_{human}$) and antimicrobial consumption in humans (AMC$_{human}$), antimicrobial consumption in animals (AMC$_{animal}$) (whether as direct or indirect influential factor), and antimicrobial resistance in bacteria in animals (AMR$_{animal}$)

Models were fitted using R plspm package (Sanchez, 2013). The non-significant relationships (p > 0.05) were discarded from the model in a step-by-step backward process.

5. Consumption of antimicrobials in humans and food-producing animals

5.1. Total tonnes of active substance and estimated biomass

Data on 2014 were chosen for analysis in this section. Data on 2013 were also analysed, and differences noted between 2013 and 2014 are commented as appropriate. Data on overall consumption for food-producing animals for 2015 were not finally approved by all reporting countries at the time when this report was finalised and were therefore excluded from the comparison of consumption in humans and animals. Summary data for 2013 can be found in Appendix D.

In 2014, 3,821 and 8,927 tonnes of active substance of antimicrobials were sold for use in humans and food-producing animals, respectively, in the 28 EU/EEA MSs delivering consumption data for both humans and animals (Table 5). In 2013, there were 26 countries delivering data for both sectors (Appendix D). The estimated biomass covered by the surveillance in 2014, expressed in 1,000 tonnes, was 31,314 for humans and 58,914 for animals, respectively. The proportion of the total biomass (sum of the biomass of food-producing animals and humans) accounted for by the animal population varied considerably between countries (from 42% to 87%). This variation, as well the different human population numbers in the EU/EEA MSs, underline the need to account for differences in population size between sectors within a country and, between countries when comparing consumption in humans and food-producing animals.

5.2. Population biomass-corrected consumption of antimicrobials in humans and food-producing animals

5.2.1. Overall consumption

The comparison of the average consumptions of antimicrobials in humans and food-producing animals (expressed in mg per kg of estimated biomass) is shown in Figure 6 and Table 5. When comparing the overall consumption of antimicrobials between the human and food-producing animal sectors in 2014, the average consumption (expressed in milligrams per kilogram of estimated biomass) equalled, respectively, 123.7 mg/kg in humans (range 49.9–181.7 mg/kg; median 118.0 mg/kg) and 151.5 mg/kg in animals (range 3.1–418.8; median 67.1 mg/kg).

The EU/EEA population-weighted mean proportion of the hospital sector AMC of the total AMC was 10%. Five countries did not report hospital sector AMC data for 2014. When interpolating these data, the EU/EEA median and the population-adjusted average (expressed as mg per kg of biomass) increased by less than 3%.

In 18 of 28 countries, the population biomass-corrected consumption was lower or much lower in food-producing animals than in humans, in two countries, the consumption was similar in both groups and in the eight remaining countries, the consumption was higher or much higher in food-producing animals than in humans. There was no correlation between the consumption in human and veterinary medicine within country (Spearman's rank correlation coefficient, $\rho = -0.04$).

5.2.2. Consumption by class

Consumption of selected antimicrobial classes, aggregated for the 28 EU/EEA MSs, is shown in Figure 7. Penicillins, macrolides and fluoroquinolones were the highest selling classes in human medicine, when expressed in milligrams per kilogram of estimated biomass. For food-producing animals, tetracyclines, penicillins and sulfonamides were the highest selling classes. Monobactams and carbapenems are not approved for use in food-producing animals in the EU/EEA MSs and no such consumption was reported in food-producing animals. Likewise, pleuromutilins are not authorised for systemic use in humans and no such consumption was reported in humans. The overall population-corrected consumption of penicillins, cephalosporins (all generations) and fluoroquinolones in humans, expressed in mg per kg of estimated biomass, was higher than the consumption of these classes in food-producing animals. For the other antimicrobial classes addressed, the opposite was the case.

Figures with data from all 28 EU/EEA MSs included can be found in Sections 6.1–10.1. The range, median and average of the consumption of the selected classes in humans and food-producing animals, expressed in mg per kg of estimated body weight, are summarised in (Table 6).

Table 5: Consumption of antimicrobials in humans and food-producing animals, in tonnes, the estimated biomass of the corresponding populations in 1,000 tonnes and consumption expressed in mg/kg biomass[a] in 28 EU/EEA MSs, 2014[b]

Country	Inclusion of 2014 consumption at the hospital	Consumption in tonnes of active substance			Estimated biomass in 1,000 tonnes			Consumption in mg/kg biomass	
		Humans	Animals	Total	Humans[c]	Animals	Total	Humans	Animals
Austria	No	38	53	91	532	948	1,480	70.9	56.3
Belgium	Yes	107	266	373	700	1,678	2,378	153.4	158.3
Bulgaria	Yes	53	33	85	453	393	846	116.0	82.9
Croatia	Yes	34	31	65	265	273	539	128.4	114.8
Cyprus	Yes	7	42	48	54	107	160	124.7	391.5
Czech Republic	No	65	56	121	657	703	1,360	99.4	79.5
Denmark	Yes	50	107	157	352	2,415	2,767	143.5	44.2
Estonia	Yes	6	10	16	82	127	210	71.7	77.1
Finland	Yes	47	11	59	341	509	850	139.2	22.3
France	Yes	717	761	1,479	4,118	7,120	11,238	174.2	107.0
Germany	No	287	1,306	1,593	5,048	8,749	13,797	56.9	149.3
Hungary	Yes	53	150	203	617	779	1,396	86.6	193.1
Iceland	No	2	1	3	20	116	136	101.7	5.2
Ireland	Yes	45	90	134	288	1,866	2,154	155.6	48.0
Italy	Yes	634	1,432	2,064	3,799	3,977	7,776	166.9	359.9
Latvia	Yes	10	6	17	125	173	298	81.6	36.7
Lithuania	Yes	19	12	31	184	335	519	102.5	35.5
Luxembourg	Yes	4	2	7	34	52	86	130.2	40.9
Netherlands	Yes	52	214	264	1,052	3,135	4,187	49.9	68.4
Norway	Yes	45	6	50	319	1,866	2,185	140.1	3.1
Poland	Yes	263	578	829	2,376	4,109	6,485	110.7	140.8
Portugal	Yes	76	190	266	652	942	1,594	116.1	201.6
Romania	Yes	226	98	323	1,247	2,502	3,749	181.7	39.1
Slovakia	Yes	47	16	64	338	248	587	140.2	65.9
Slovenia	Yes	14	6	19	129	171	300	105.5	33.4
Spain	No	327	2,964	3,291	2,907	7, 077	9,984	112.6	418.8
Sweden	Yes	72	9	82	603	811	1,414	119.8	11.5
United Kingdom	Yes	518	430	939	4,022	6,915	10,937	128.7	62.1
All[a]		3,821	8,927	12,720	31,314	58,914	90,228	123.7[d]	151.5

(a): Calculated from the exact figures (not rounded as shown).

(b): The estimates presented are crude and must be interpreted with caution. Countries with less than 95% data coverage for community consumption in humans were Germany (85%) and the Netherlands (92%). In those countries, the consumption expressed in tonnes, without correction for population or biomass, will be an underestimate. For further limitations that may hamper the comparison of the consumptions of antimicrobials in humans and in animals, please see Section 14.

(c): Population covered by data in ESAC-Net.

(d): Population weighted mean.

Figure 6: Comparison of biomass-corrected consumption of antimicrobials (mg/kg of estimated biomass) in humans and food-producing animals by country, EU/EEA MSs, 2014
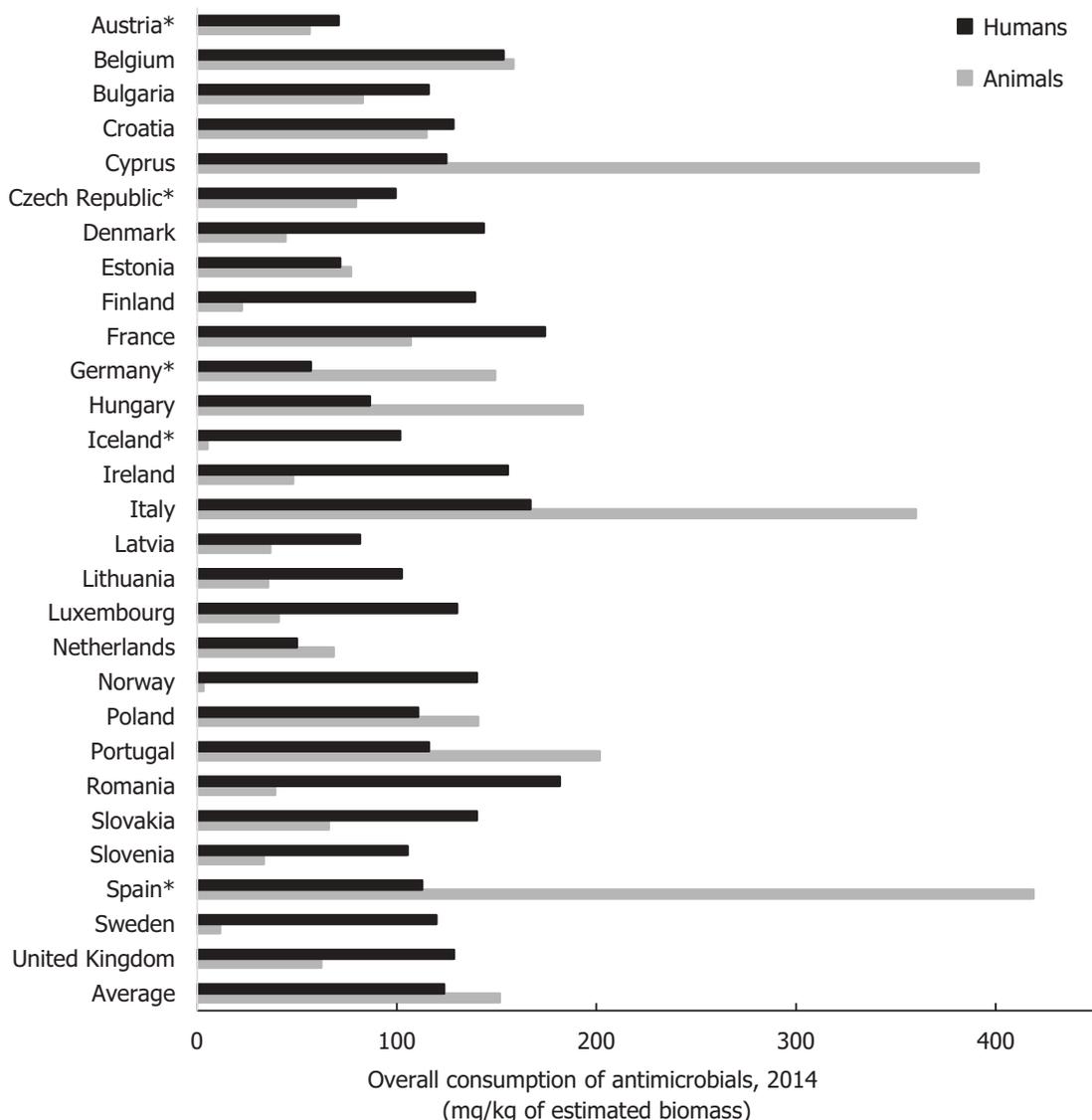
Asterisk (*) denotes that only community consumption was provided for human medicine. The population-weighted mean proportion (%) of the hospital sector AMC of the 2014 total national AMC for EU/EEA MSs that provided data for both sectors is 10%.

Note: 1) The estimates presented are crude and must be interpreted with caution. For limitations that hamper the comparison of consumption of antimicrobials in humans and animals, please see Section 14.

2) The average figure represents the population-weighted mean of data from included countries.

Notes: 1) The y-axis scale differs between the graphs A, B and C.

2) The estimates presented are crude and must be interpreted with caution. For limitations that hamper the comparison of consumption of antimicrobials in humans and animals, please see Section 14.

3) Classes not included for human medicine were monobactams (ATC group J01DF), other cephalosporins and penems (J01DI), streptogramins (J01 FG), glycopeptides, imidiazoles, nitrofurans, steroid antimicrobials and other antimicrobials (J01XX). Substances not included for food-producing animals were bacitracin (ATCvet group QA07AA93 and QJ01XX10), paromomycin (QJ01GB92) and spectinomycin (QJ01XX04).

Figure 7: Comparison of consumption of selected antimicrobial classes in humans and food-producing animals, EU/EEA MSs, 2014

Table 6: Range, median and population-weighted average of the consumption of the antimicrobial classes selected for analysis in humans and food-producing animals in 28 EU/EEA MSs in 2014, expressed in mg/kg estimated body weight and results of Spearman's rank correlation analysis of consumption in animals and humans within country

Antimicrobial class	Humans			Animals			$\rho^{(b)}$ (p-value)
	Range	Median	Average[a]	Range	Median	Average	
Third- and fourth-generation cephalosporins	< 0.01–12.1	2.0	3.8	< 0.01–0.8	0.2	0.2	0.22 (0.251)
Fluoroquinolones and other quinolones	3.1–17.4	6.2	8.1	0–11.6	1.7	3.5	0.56 (0.002)
Polymyxins	0–0.1	0.01	0.03	0–36.1	1.3	10.0	0.30 (0.122)
Macrolides	1.5–19.8	6.5	7.8	0–27.5	4.9	11.4	0.32 (0.100)
Tetracyclines	0.3–13.5	1.8	3.6	0.1–151.5	25.1	50.6	−0.035 (0.058)

(a): Population weighted mean.
(b): Spearman's rank correlation coefficient.

5.3. Key findings on the comparison of consumption

- The overall average consumption of antimicrobials, expressed in milligrams per kilogram of estimated biomass and per year, was lower in humans than in food-producing animals; in contrast, the median was higher in humans. A limited number of countries with significant animal populations and a comparatively high consumption had a large influence on the average.
- In 18 of the 28 countries studied, the consumption of antimicrobials was lower or much lower in food-producing animals than in humans, in two countries, the consumption was similar in the two groups and in the eight remaining countries, the consumption was higher or much higher in food-producing animals than in humans.
- Overall, penicillins, macrolides and fluoroquinolones were the most consumed antimicrobial classes in human medicine, when consumption is expressed in milligrams per kilogram of estimated biomass, whereas in veterinary medicine, tetracyclines, penicillins and sulfonamides were the most used antimicrobial classes.
- No correlation was observed between overall AMC in animals and in humans at the country level.

6. 3rd- and 4th-generation cephalosporins

6.1. Consumption of 3rd- and 4th-generation cephalosporins by country

In 2014, the average consumption (population-weighted mean) of 3rd- and 4th-generation cephalosporins in humans and food-producing animals was 3.8 and 0.2 mg/kg of estimated biomass, respectively (Figure 8). The corresponding ranges were < 0.1–12.1 (median 2) and < 0.1–0.8 (median 0.2) mg/kg, respectively. In Figure 8, the biomass-corrected consumption in humans and food-producing animals is shown by country.

The consumption of 3rd- and 4th-generation cephalosporins in food-producing animals was, with one exception, much lower than that reported in human medicine. In the country in question, the consumption was negligible both in animals and humans. It is also of note that this country did not report on hospital consumption.

There was no correlation between the consumption of 3rd- and 4th-generation cephalosporins in humans and in food-producing animals (Spearman's rank correlation coefficient, $\rho = 0.22$) at the country level.
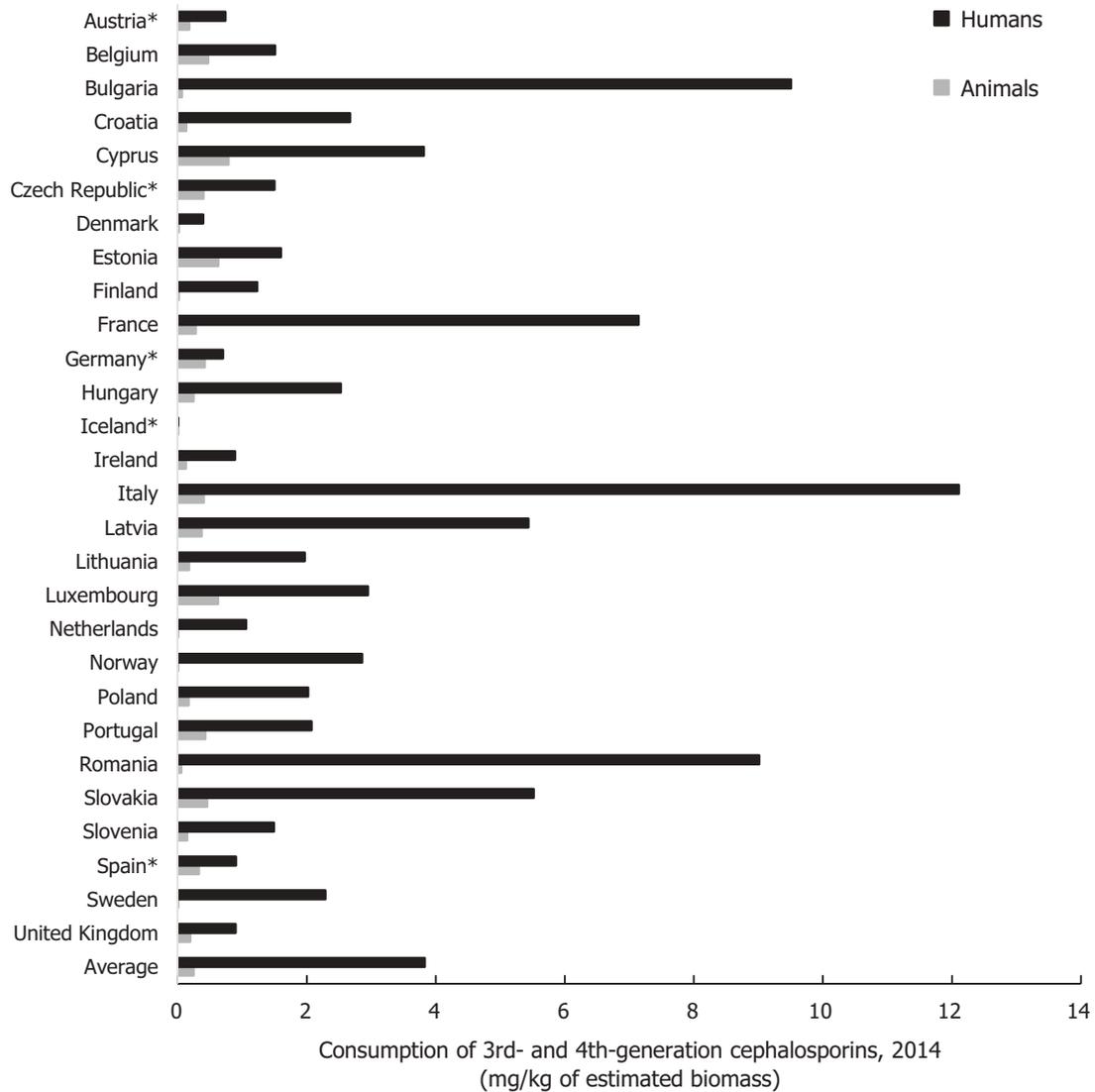
Asterisk (*) denotes that only community consumption was provided for human medicine. The population-weighted mean proportion (%) of the hospital sector from the 2014 total national consumption of antimicrobials for EU/EEA MSs that provided data for both sectors was 51.1%.

1) The estimates presented are crude and must be interpreted with caution. For limitations that hamper the comparison of consumption of antimicrobials in humans and animals, please see Section 14.

2) The average figure represents the population-weighted mean of data from included countries.

Figure 8: Biomass-corrected consumption of 3rd- and 4th-generation cephalosporins in humans and food-producing animals by country, EU/EEA MSs, 2014

6.2. Consumption of 3rd- and 4th-generation cephalosporins for humans and occurrence of resistance to 3rd-generation cephalosporins in bacteria from humans

Third- and 4th-generation cephalosporins (primarily 3rd-generation) are used for the treatment of infections caused by both Gram-positive and Gram-negative bacteria, including infections caused by *E. coli* and *S.* Enteritidis, *S.* Typhimurium, monophasic *S.* Typhimurium and *S.* Infantis. Third- and 4th-generation cephalosporins are considered by WHO as highest priority CIAs which should be reserved for the treatment of severe infections in humans (5th revision—WHO list of critically important antimicrobials (CIA)) (WHO, 2017), indicating that non-human use of these antimicrobials should be avoided when possible. In accordance, AMEG has classified 3rd- and 4th-generation cephalosporins in Category 2 which means that these antimicrobials should be used in veterinary medicine only when there are no alternative antimicrobials authorised for the respective target species and indication.

6.2.1. Invasive *E. coli* from cases of human infection

Data on occurrence of cephalosporin resistance in invasive *E. coli* from humans were reported by 28 EU and two EEA MSs in 2013, 2014 and 2015. Potential association between the consumption of 3rd- and 4th-generation cephalosporins and the occurrence of resistance to 3rd-generation cephalosporins in invasive *E. coli* isolates from humans was assessed for the years 2013, 2014 and 2015 (Table 7). For each of the 3 years, significant (p < 0.01) positive association between resistance of invasive *E. coli* isolates from humans and consumption of 3rd- and 4th-generation cephalosporins in EU/EEA, was observed. A higher consumption (by 1 unit DDD) of 3rd- and 4th-generation cephalosporins was associated with a higher probability of resistance (87%) in invasive *E. coli* isolates from humans in 2013. Similarly, in both 2014 and 2015, a higher consumption was associated with a two-fold higher probability of resistance.

When comparing consumption of 3rd- and 4th-generation cephalosporins in the community and at the hospital separately with the occurrence of resistance to 3rd-generation cephalosporins in invasive *E. coli* from humans, significant associations were also detected in both cases. In this analysis, significant association within the hospital sector had high OR in contrast to low OR found in the community sector. Some extreme values may have resulted in high OR in hospital sector (data not shown).

Table 7: Results of logistic regression for total (community and hospital) consumption of 3rd- and 4th-generation cephalosporins in humans, expressed in DDD per 1,000 inhabitants and per day, and the probability of resistance to 3rd-generation cephalosporins in invasive *E. coli* from humans, EU/EEA, 2013–2015 (see also Figure 9)

Year	Countries included in the analysis	OR	95% PL CI	p-value
Invasive *E. coli*				
2013	AT, BE, BG, CY, CZ, DE, DK, EE, EL, ES, FI, FR, HR, HU, IE, IS, IT, LT, LU, LV, MT, NL, NO, PL, PT, RO, SE, SI, SK, UK (n = 30)	1.87	1.32–2.65	0.001
2014	AT, BE, BG, CY, CZ, DE, DK, EE, EL, ES, FI, FR, HR, HU, IE, IS, IT, LT, LU, LV, MT, NL, NO, PL, PT, RO, SE, SI, SK, UK (n = 30)	2.03	1.50–2.73	< 0.001
2015	AT, BE, BG, CY, CZ, DE, DK, EE, EL, ES, FI, FR, HR, HU, IE, IS, IT, LT, LU, LV, MT, NL, NO, PL, PT, RO, SE, SI, SK, UK (n = 30)	1.94	1.47–2.54	< 0.001

OR: odds ratio; 95% PL CI: profile likelihood confidence interval, 95%.
OR varies from 0 to infinity. When OR equals 1 or CI includes 1, the association is not considered statistically-significant.

1) Consumption of cephalosporins and resistance in *E. coli* from humans, 2013

2) Consumption of cephalosporins and resistance in *E. coli* from humans, 2014

3) Consumption of cephalosporins and resistance in *E. coli* from humans, 2015
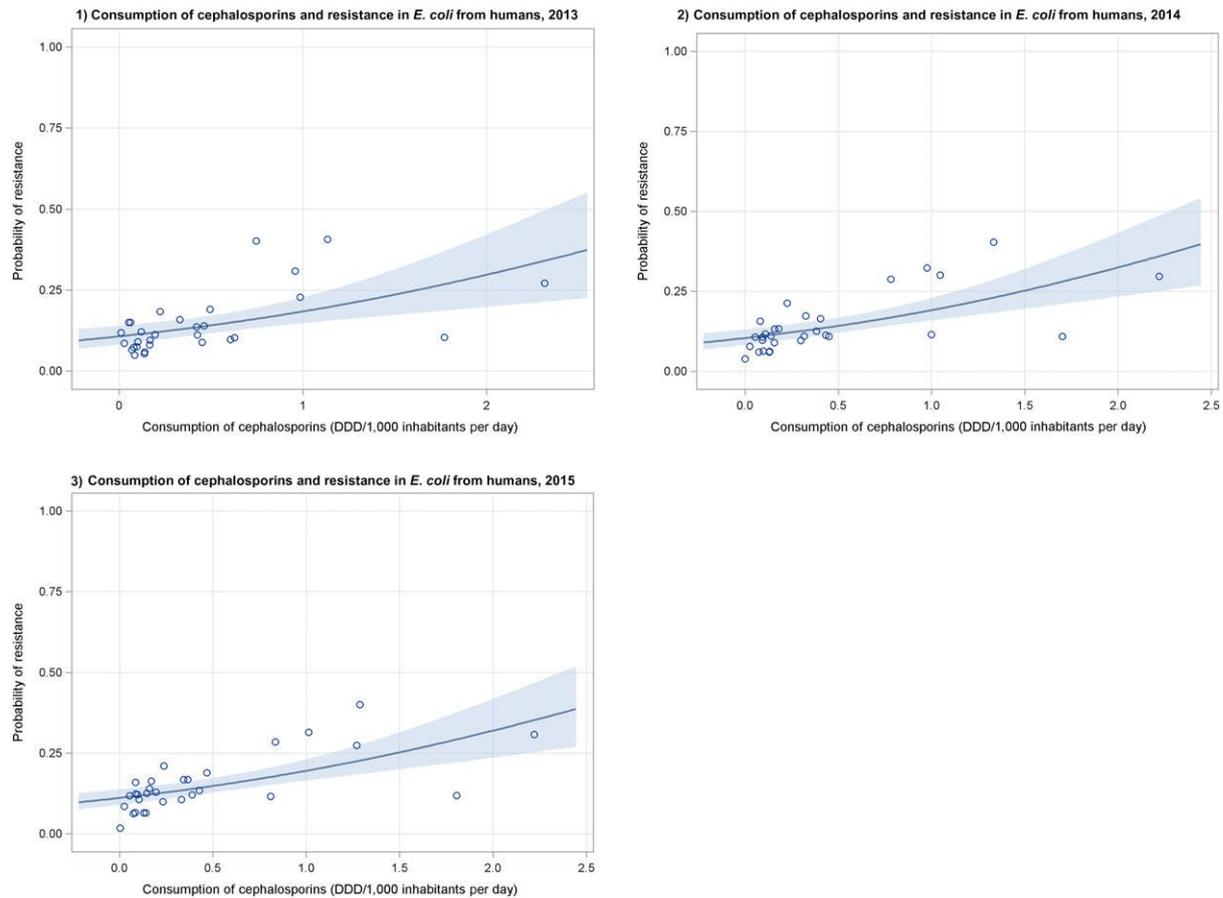
Dots represent countries included in the analysis.

Figure 9: Logistic regression analysis curves of the total (community and hospital) consumption of 3rd- and 4th-generation cephalosporins in humans, expressed in DDD per 1,000 inhabitants and per day, and the probability of resistance to 3rd-generation cephalosporins in invasive *E. coli* from humans, EU/EEA, (1) 2013, (2) 2014 and (3) 2015 (see also Table 7)

6.2.2. *S.* Enteritidis, *S.* Typhimurium, monophasic *S.* Typhimurium and *S.* Infantis

Data on occurrence of resistance to 3rd-generation cephalosporins in *S.* Enteritidis were reported by 16 countries in 2013, 17 countries in 2014 and 19 countries in 2015 and in *S.* Typhimurium by 18 countries in 2013 and 2014, and by 20 countries in 2015. A lower number of countries provided data on resistance for *S.* Infantis (13 countries in 2013, 12 countries in 2014 and 10 countries in 2015) and monophasic *S.* Typhimurium (10 countries in 2013, 10 countries in 2014 and 11 countries in 2015).

For the years 2013 and 2014, significant associations between resistance to 3rd-generation cephalosporins and total (community and hospital) or community consumption alone, of 3rd- and 4th-generation cephalosporins, were observed only for *S.* Infantis (Table 8, Figure 10). Because of the limited number of countries included in the analysis of *S.* Infantis, these results should not be extrapolated to the EU/EEA level. Resistance to 3rd-generation cephalosporins of *S.* Enteritidis, *S.* Typhimurium and monophasic *S.* Typhimurium for the same time period (2013–2014) was not correlated either to the total (community and hospital) consumption or to community consumption only of 3rd- and 4th-generation cephalosporins in humans (Table 8).

When omitting the country with the large value in 2013 and 2014 (upper right corner) in the sensitivity analysis of the *S.* Infantis results, the correlation became non-significant.

Table 8: Results of logistic regression for total (community and hospital) consumption of 3rd- and 4th-generation cephalosporins in humans, expressed in DDD per 1,000 inhabitants and per day, and the probability of resistance to 3rd-generation cephalosporins in selected salmonella serovars from humans, EU/EEA, 2013–2015 (see also Figures 10 and 11)

Year	Countries included in the analysis	OR	95% PL CI	p-value
_S._ Typhimurium				
2013	AT, BE, DE, DK, EE, ES, FI, FR, HU, IE, IT, LT, LU, NL, RO, SI, SK, UK (n = 18)	0.60	0.18–1.40	0.314
2014	AT, BE, DE, DK, ES, FI, FR, HU, IE, LT, LU, NL, NO, PT, RO, SI, SK, UK (n = 18)	1.38	0.25–4.49	0.649
2015	AT, DE, DK, EE, EL, ES, FI, FR, HU, IE, IT, LT, LU, NL, NO, PT, RO, SI, SK, UK (n = 20)	0.06	0.01–0.58	0.052
Monophasic _S._ Typhimurium				
2013	AT, DK, ES, FR, HU, IE, IT, LU, NL (n = 9)	0.47	0.18–0.92	0.061
2014	AT, DK, ES, FR, HU, IE, IT, LU, NL, PT (n = 10)	1.15	0.28–3.02	0.804
2015	AT, DK, EE, ES, FR, HU, IE, IT, LU, NL, PT (n = 11)	0.68	0.10–2.09	0.602
_S._ Enteritidis				
2013	AT, BE, DE, EE, ES, FI, HU, IE, IT, LT, LU, NL, RO, SI, SK, UK (n = 16)	1.12	0.07–4.80	0.908
2014	AT, BE, DE, ES, FI, FR, HU, IE, LT, LU, NL, NO, PT, RO, SI, SK, UK (n = 17)	1.78	0.19–8.96	0.542
2015	AT, DE, EE, EL, ES, FI, FR, HU, IE, IT, LT, LU, NL, NO, PT, RO, SI, SK, UK (n = 19)	3.77	0.93–10.80	0.028
_S._ Infantis				
2013	AT, BE, DE, ES, FI, IE, IT, LT, NL, RO, SI, SK, UK (n = 13)	4.01	1.66–9.79	0.001
2014	AT, BE, DE, DK, ES, HU, IT, LT, NL, SI, SK, UK (n = 12)	6.84	3.58–13.87	< 0.001
2015	AT, DE, EE, ES, FI, HU, LT, NL, SI, UK (n = 10)	1.60	0.05–646.93	0.878

OR: odds ratio; PL CI: profile likelihood confidence interval, 95%.
OR varies from 0 to infinity. When OR equals 1 or CI includes 1, the association is not considered statistically-significant.
Exceptions may occur when the over-dispersion correction proposed by Williams is used.
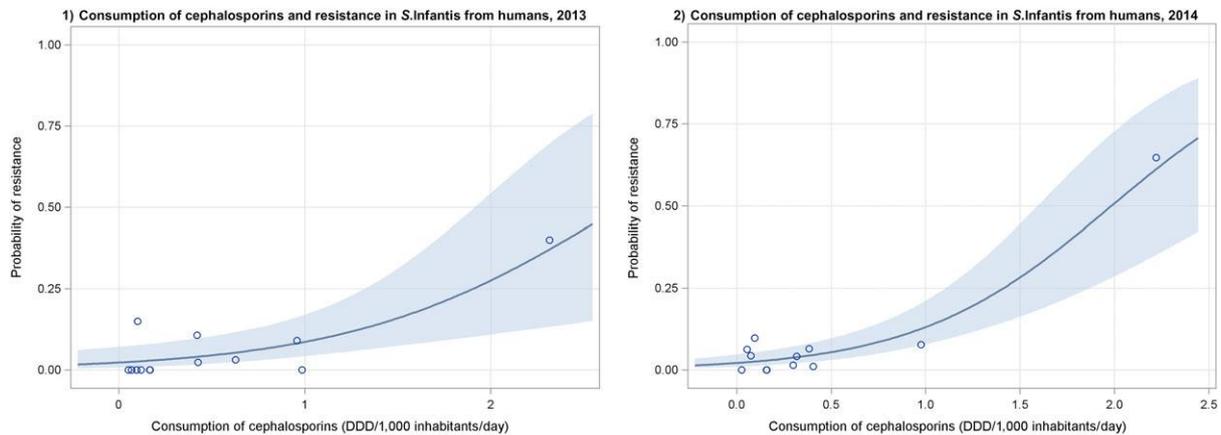
Dots represent countries included in the analysis.

Figure 10: Logistic regression analysis curves of the total (community and hospital) consumption of 3rd- and 4th-generation cephalosporins in humans, expressed in DDD per 1,000 inhabitants, and per day and the probability of resistance to 3rd-generation cephalosporins in _S._ Infantis isolates from humans, EU/EEA, (1) 2013 and (2) 2014 (see also Table 8)

Consumption to cephalosporins and resistance in *S.* Enteritidis from humans, 2015
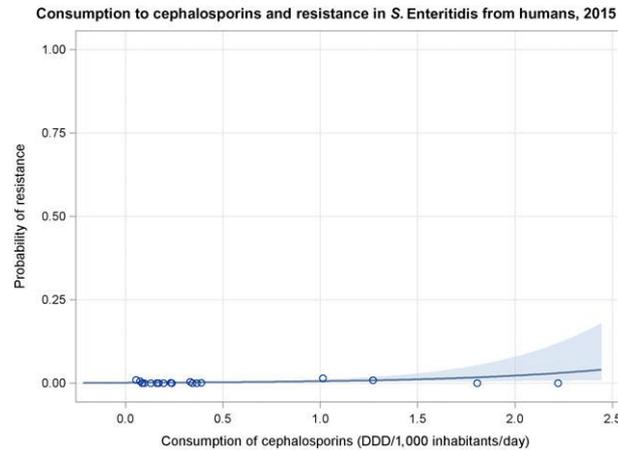
Dots represent countries included in the analysis.

Figure 11: Logistic regression analysis curves of the total (community and hospital) consumption of 3rd- and 4th-generation cephalosporins in humans, expressed in DDD per 1,000 inhabitants and per day, and the probability of resistance to 3rd-generation cephalosporins in *S.* Enteritidis from humans, EU/EEA, 2015 (see also Table 8)

6.3. Comparison of consumption of 3rd- and 4th-generation cephalosporins in animals with resistance to cefotaxime in bacteria from animals

6.3.1. In food-producing animals

In order to investigate possible relationships between the consumption of 3rd- and 4th-generation cephalosporins and cephalosporin resistance, the SIMR to cefotaxime in indicator *E. coli* and *Salmonella* spp. from food-producing animals was compared with the consumption of 3rd- and 4th-generation cephalosporin in animals (expressed in mg per kg of estimated biomass) for 2013 and 2014–2015 (average consumption over 2014 and 2015) at the country level (Table 9). The category 'food-producing animals' includes broilers, pigs and cattle for 2013 and broilers, turkeys, pigs and calves for 2014–2015.

Although some disparity in consumption of 3rd- and 4th-generation cephalosporins was recorded among the countries considered, cefotaxime resistance in both types of bacteria was typically reported at low to very low levels or was undetected. Although positive associations between cefotaxime resistance in indicator *E. coli* and *Salmonella* spp. and the consumption of 3rd- and 4th-generation cephalosporins in animals were observed in 2013 and 2014–2015, only the association assessed in indicator *E. coli* in 2014–2015 was statistically-significant. In this latter case, sensitivity analysis showed that the association did not remain significantly positive after ignoring the point displayed in the upper right corner of the graph: p-value > 0.05, OR = 1.27; 95% PL CI: 0.89–1.84.

Table 9: Results of logistic regression for consumption of 3rd- and 4th-generation cephalosporins in food-producing animals, expressed in mg/kg of estimated biomass/year, and the probability of resistance to 3rd-generation cephalosporins in bacteria from food-producing animals (see also Figure 12)

Year	Countries included in the analysis	OR[a]	95% PL CI	p-value
Indicator *E. coli*				
2013[b]	AT, BE, CH, DE, DK, ES, FI, HU, NL, PL, SE (n = 11)	1.30	0.86–1.99	0.210
2014–2015	AT, BE, BG, CY, CZ, DE, DK, EE, ES, FI, FR, HR, HU, IE, IT, LT, LV, NL, NO, PL, PT, RO, SE, SI, SK, UK (n = 26)	1.31	1.03–1.68	0.026
***Salmonella* spp.**				
2013[b]	BE, DE, DK, ES, IE, IT, NL, PL, UK (n = 9)	1.14	0.63–2.08	0.250
2014–2015	BE, CZ, DK, ES, FR, HR, IT, PT (n = 8)	3.01	1.18–15.9	0.015

OR: odds ratio; PL CI: profile likelihood confidence interval, 95%.
OR varies from 0 to infinity. When OR equals 1 or CI includes 1, the association is not considered statistically-significant.
Regarding resistance data, the category 'food-producing animals' includes broilers, pigs and cattle for 2013, and broilers, turkeys, pigs and calves for 2014–2015.
(a): OR estimated for 0.1-unit increment.
(b): In the absence of 2013 resistance data, proxy data for years prior to 2013 may have been used.

Dots represent the countries involved in the analysis. The category 'food-producing animals' includes broilers, pigs and cattle for 2013, and broilers, turkeys, pigs and calves for 2014–2015. The scale used in these graphs is adapted according to the range of probabilities of resistance observed, in order to best show the distribution of data points.

Figure 12: Logistic regression analysis curves of the consumption of 3rd- and 4th-generation cephalosporins in food-producing food-producing animals and the probability of resistance to cefotaxime in (1) indicator *E. coli* and (2) *Salmonella* spp. from food-producing animals, 2014–2015 (see also Table 9)

6.3.2. In pigs

The estimated consumption of 3rd- and 4th-generation cephalosporins in pigs was compared with the occurrence of resistance to cefotaxime in indicator *E. coli* from slaughter pigs for the years 2013 and 2015 for 11 and 18 reporting countries, respectively.

Although variations in consumption of 3rd- and 4th-generation cephalosporins were observed among the countries considered, cefotaxime resistance in indicator *E. coli* from slaughter pigs was typically reported at very low levels. None of the associations assessed for the years 2013 and 2015 were statistically-significant, although found to be positive and of the same magnitude (Table 10).

Table 10: Results of logistic regression for the estimated consumption of 3rd- and 4th-generation cephalosporins in pigs, expressed in DDDvet/kg of estimated biomass/year, and the probability of resistance to cefotaxime in indicator *E. coli* from slaughter pigs

Animal	Year	Countries included in the analysis	OR[a]	95% PL CI	p-value
Indicator *E. coli*					
Slaughter pigs	2013[b]	AT, BE, CH, DE, DK, ES, FI, HU, NL, PL, SE (n = 11)	1.22	0.70–2.14	0.481
	2015	AT, BE, BG, CH, CY, DE, EE, FI, HR, HU, IE, LV, NO, PL, PT, RO, SE, SI (n = 18)	1.20	0.66–1.95	0.523

OR: odds ratio; PL CI = profile likelihood confidence interval, 95%.
OR varies from 0 to infinity. When OR equals 1 or CI includes 1, the association is not considered statistically-significant.
(a): OR estimated for 0.1-unit increment.
(b): In the absence of 2013 resistance data, proxy data for years prior to 2013 may have been used.

The corresponding analysis was not performed in poultry, since there is no veterinary product based on 3rd-generation cephalosporins authorised in poultry.

6.4. Resistance to 3rd- and 4th-generation cephalosporins in bacteria from animals *versus* resistance in bacteria from humans

6.4.1. Resistance in indicator *E. coli* from food-producing animals and in invasive *E. coli* from humans

Data on the occurrence of resistance of invasive *E. coli* from humans to 3rd-generation cephalosporins (2013–2015) were compared with the occurrence of resistance to 3rd-generation cephalosporins of indicator *E. coli* from pigs and cattle (2013 and 2015) as well as from broilers and turkeys (2013–2014). No significant association for resistance in animals and humans were found for the above mentioned combinations (Table 11).

When combining data for 2014 and 2015 of resistance to 3rd-generation of cephalosporins of invasive *E. coli* from humans and of indicator *E. coli* from food-producing animals (SIMR) no significant correlation was observed (Table 11). When two countries (outliers) were excluded from the analysis a significant correlation was found (OR = 1.07, p = 0,008) (Figure 13).

Table 11: Results of logistic regression for the probability of resistance to 3rd- and 4th-generation cephalosporins in invasive *E. coli* from humans and resistance to 3rd-generation cephalosporins in indicator *E. coli* from food-producing animals (see also Figure 13)

Animal	Year	Countries	OR	95% PL CI	p-value
E. coli					
FPA	2014–2015	AT, BE, BG, CY, CZ, DE, DK, EE, ES, FI, FR, HR, HU, IE, IT, LT, LV, NL, NO, PL, PT, RO, SE, SI, SK, UK (n = 26)	1.57	0.73–3.17	0.228
Broilers	2013	AT, BE, DE, DK, ES, FI, FR, HR, HU, NL, PL, SE (n = 12)	1.03	0.99–1.06	0.170
	2014	AT, BE, BG, CY, CZ, DE, DK, EE, EL, ES, FI, FR, HR, HU, IE, IT, LT, LV, MT, NL, NO, PL, PT, RO, SE, SI, SK, UK (n = 28)	1.08	0.98–1.04	0.567
Turkeys	2014	AT, DE, ES, FR, HU, IT, PL, PT, RO, SE, UK (n = 11)	0.95	0.80–1.10	0.550
Pigs	2013	AT, BE, DE, DK, ES, FI, FR, HR, HU, NL, PL, SE, UK (n = 13)	1.11	0.92–1.31	0.263
	2015	AT, BE, BG, CY, CZ, DE, DK, EE, EL, ES, FI, FR, HR, HU, IE, IT, LT, LV, MT, NL, NO, PL, PT, RO, SE, SI, SK, UK (n = 28)	1.05	0.91–1.20	0.528

FPA: food-producing animals; OR: odds ratio; PL CI: profile likelihood confidence interval, 95%.
OR varies from 0 to infinity. When OR equals 1 or CI includes 1, the association is not considered statistically-significant.

Resistance to cephalosporins in *E. coli* fromfood-producing
animals and from humans, 2014–2015
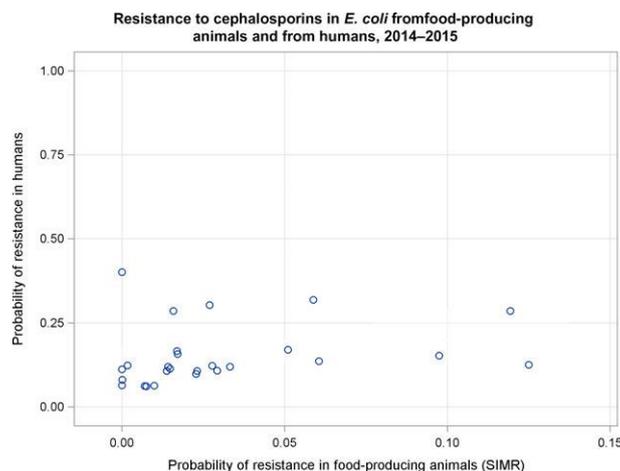
Dots represent countries included in the analysis. Note: The figure displays a non-significant correlation. When two countries (outliers) were excluded from the analysis, a significant correlation was found.

Figure 13: Logistic regression analysis curves of the probability of resistance to 3rd-generation cephalosporins in invasive *E. coli* from humans and the probability of resistance in indicator *E. coli* (SIMR) from food-producing animals (combined data for 2014–2015) (see also Table 11)

6.4.2. Resistance in *Salmonella* Enteritidis, *S*. Typhimurium, and *S*. Infantis from humans and food-producing animals

For *S.* Typhimurium, in 2013, six countries provided both data for human infection and pigs and analysis including these countries showed no significant correlation (Table 12).

Table 12: Results of logistic regression for probability of resistance to 3rd- and 4th-generation cephalosporins in *S.* Typhimurium from slaughter pigs and humans

| Animal	Year	Countries	OR	95% PL CI	p-value
S. **Typhimurium**					
Slaughter pigs	2013	BE, DE, DK, IE, NL, UK (n = 6)	1.20	0.72–2.01	0.473

OR: odds ratio; PL CI: profile likelihood confidence interval, 95%.
OR varies from 0 to infinity. When OR equals 1 or CI includes 1, the association is not considered statistically-significant.

For *S*. Enteritidis and *S*. Infantis, there were either an insufficient number of countries providing data from both sectors or no resistance had been detected in animals. One exception was high cefotaxime resistance in broilers and humans in one country most likely due to spread of a clone of ESBL-producing *S*. Infantis. Using aggregate data for 2014 and 2015, resistance of *Salmonella* spp. to 3rd-generation cephalosporins from humans significantly correlated to resistance of *Salmonella* spp. to 3rd-generation cephalosporins from food-producing animals (SIMR). When one extreme value was omitted, however, a significant negative correlation was observed, indicating that the results were not reliable due to the low number of countries included and low resistance observed.

6.5. Consumption of 3rd- and 4th-generation cephalosporins in food-producing animals versus resistance in bacteria from humans

In order to investigate a possible relationship between the consumption of 3rd- and 4th-generation cephalosporins in food-producing animals with data on AMR in bacteria causing infections in humans, the occurrence of AMR in *E. coli* and *Salmonella* spp. from humans was compared with consumption of 3rd- and 4th-generation cephalosporins in food-producing animals (expressed in milligrams per kilogram of estimated biomass) in 2013, 2014 and 2015. Although positive associations between 3rd- and 4th-generation cephalosporin resistance in *E. coli* BSI and *Salmonella* spp. from humans and consumption of 3rd- and 4th-generation cephalosporins in food-producing animals was observed in 2013, 2013 and 2015, none were statistically-significant (Table 13).

Table 13: Results of logistic regression for consumption of 3rd- and 4th-generation cephalosporins in food-producing animals, expressed in mg/kg of estimated biomass/year, and the probability of resistance to 3rd- and 4th- generation cephalosporins in bacteria causing infections in humans

Year	Countries included in the analysis	OR[a]	95% PL CI	p-value
Invasive *E. coli*				
2013	AT, BE, BG, CY, CZ, DE, DK, EE, ES, FI, FR, HU, IE, IS, IT, LT, LU, LV, NL, NO, PL, PT, SE, SI, SK, UK (n = 26)	2.72	0.53–13.81	0.229
2014	AT, BE, BG, CY, CZ, DE, DK, EE, ES, FI, FR, HR, HU, IE, IS, IT, LT, LU, LV, NL, NO, PL, PT, RO, SE, SI, SK, UK (n = 28)	2.75	0.77–9.57	0.117
2015	AT, BE, BG, CY, DE, DK, EE, FI, FR, HR, HU, IE, IS, IT, LV, NL, NO, PL, PT, RO, SE, SI, UK (n = 23)	4.13	0.78–21.8	0.094
***Salmonella* spp.**				
2013	AT, BE, DE, DK, EE, ES, FI, FR, HU, IE, IT, LT, LU, NL, SI, SK, UK (n = 17)	1.16	0.94–1.41	0.175
2014	AT, BE, DE, DK, EE, ES, FI, FR, HU, IE, IT, LT, LU, NL, NO, PT, RO, SI, SK, UK (n = 20)	1.10	0.79–1.52	0.551
2015	AT, CY, DE, DK, EE, FI, FR, HU, IE, IT, NL, NO, PT, RO, SI, UK (n = 16)	1.01	0.75–1.33	0.946

OR: odds ratio; PL CI: profile likelihood confidence interval, 95%.
OR varies from 0 to infinity. When OR equals 1 or CI includes 1, the association is not considered statistically-significant.
(a): OR estimated for 0.1-unit increment.

6.6. Multivariate analysis

The only significant relationship retained in the final model of resistance to 3rd-generation cephalosporins in invasive *E. coli* from humans related to the strong (p < 0.0001) direct effect of the consumption of 3rd- and 4th-generation cephalosporins in humans, for which the consumption at the hospital seemed to have the greatest weight (0.798) (Figure 14).

For *Salmonella* spp., the data was limited to 11 countries and no significant relationship could be identified for any of the associations.
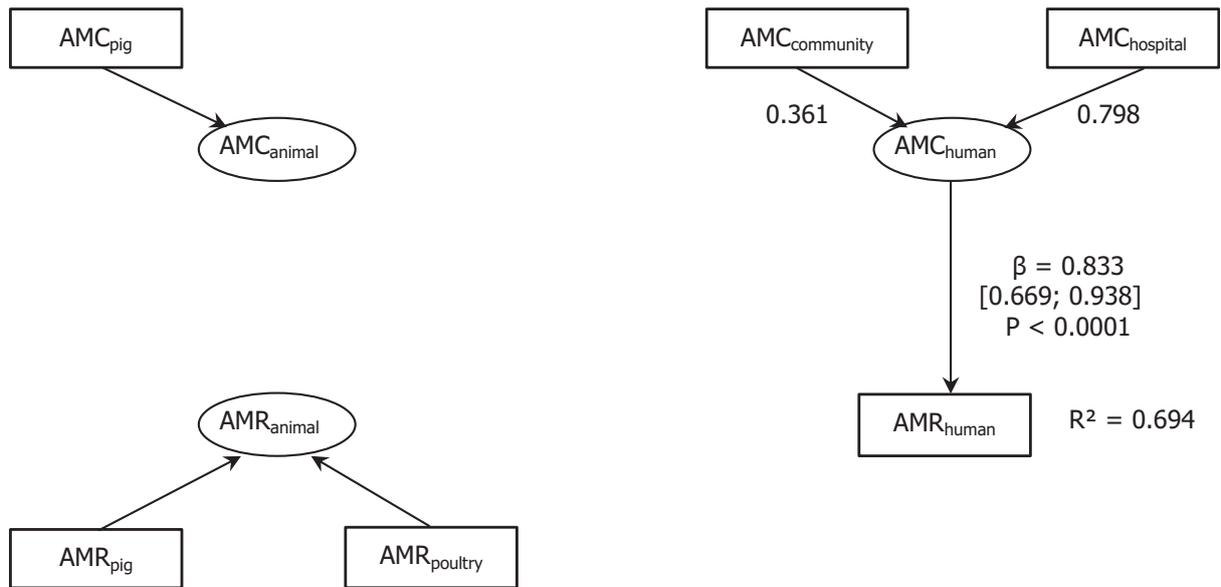
26 countries: AT*, BE, BG, CY, CZ*†, DE*, DK, EE, ES*†, FI, FR, HR, HU, IE, IT, LT†, LV, NL, NO, PL, PT, RO, SE, SI, SK†, UK (goodness-of-fit = 0.686).
†For these countries, the AMC in pigs in 2014 was used as a surrogate of that for 2015 (missing data).
*For these countries, the AMC at the hospital was estimated.

Figure 14: PLS-PM model of resistance to 3rd-generation cephalosporins in human invasive *E. coli* (2014–2015) considering resistance to 3rd-generation cephalosporins in indicator *E. coli* from animals (pigs in 2015 and poultry in 2014), consumption of 3rd- and 4th-generation cephalosporins in humans (average in 2014–2015, expressed in DDD per 1,000 inhabitants and per day) and in animals (in pigs in 2015, expressed in DDDvet/kg of estimated biomass)

6.7. Key findings on 3rd- and 4th-generation cephalosporins

- In human medicine, 3rd- and 4th-generation cephalosporins are generally primarily used in hospitals. In countries where consumption data were only available in the community, the consumption of 3rd- and 4th-generation cephalosporins was therefore considerably underestimated. AMEG has classified 3rd- and 4th-generation cephalosporins in category 2, implying that restriction of use in animals is needed (EMA/AMEG, 2014). The consumption of 3rd- and 4th-generation cephalosporins in food-producing animals was much lower than that observed in humans. No statistically-significant correlation was observed between consumption in humans and in food-producing animals at the country level.

- In humans, the consumption of 3rd- and 4th-generation cephalosporins in the community, at the hospital or in total (community and hospital) was significantly and positively associated with resistance to 3rd-generation cephalosporins in invasive *E. coli* from humans. In all four non-typhoidal salmonella serovars (*S.* Enteritidis, *S.* Typhimurium, monophasic *S.* Typhimurium and *S.* Infantis) studied from humans, resistance was in general very low in all study years. Significant positive associations between the consumption of 3rd- and 4th-generation cephalosporins in humans and resistance to this sub-class were inconsistently detected in some serovars (*S.* Enteritidis and *S.* Infantis) only.

- In food-producing animals, overall, no association was observed (with a few exceptions) between consumption of and resistance to 3rd- and 4th-generation cephalosporins. Associations between AMC and AMR were assessed using available data on the occurrence of phenotypic resistance to 3rd- and 4th-generation cephalosporins. Investigation of these associations in specific animal sub-populations, and accounting for resistance genotypes would have helped in further refining the analysis, but could not be performed because of lack of data.

- No statistically-significant association was observed between resistance to 3rd-generation cephalosporins in *E. coli* and *Salmonella* spp. from food-producing animals and humans, when analysing data for each year and per animal species. The resistance to 3rd- and 4th-generation

cephalosporins in indicator *E. coli* from food-producing animals for 2014–2015, assessed by the SIMR, was significantly and positively associated with resistance to 3rd-generation cephalosporins in invasive *E. coli* from humans, after outliers were excluded.

- The consumption of 3rd- and 4th-generation cephalosporins in food-producing animals was not associated with resistance to 3rd-generation cephalosporins in either invasive *E. coli* or *Salmonella* spp. from humans.
- The multivariate analysis showed that the only significant relationship retained in the final model of resistance to 3rd- and 4th-generation cephalosporins in invasive *E. coli* from humans was the strong direct impact of the consumption of these classes of antimicrobials in humans.

7. Fluoroquinolones and other quinolones

7.1. Consumption of fluoroquinolones and other quinolones by country

Quinolones (fluoroquinolones and other quinolones) are regarded by the WHO as CIAs of highest priority (5th revision—WHO list of critically important antimicrobials (CIA)) (WHO, 2017)). In 2014, the population-weighted mean consumption of fluoroquinolones in humans and food-producing animals was 8.0 and 2.9 mg per kg of estimated biomass, respectively. The corresponding ranges were 3.1–17.1 (median 6.2) and 0–11.4 (median 1.5) mg per kg of estimated biomass, respectively. Average, range and median were similar for fluoroquinolones and other quinolones (Table 6), except that the average consumption in animals that was 3.5 mg per kg of estimated biomass. Population-corrected consumption of fluoroquinolones and other quinolones in humans and food-producing animals by country is shown in Figure 15.

Overall, in most countries, the consumption of fluoroquinolones was lower in food-producing animals than in humans. In three countries, the consumption was higher in animals than in humans. Also, the variation between countries in the quantity of fluoroquinolones consumed in humans or animals was very marked. There was a significant correlation within country between the consumption of fluoroquinolones and that of fluoroquinolones and other quinolones in humans and food-producing animals (Spearman's rank correlation coefficients: $\rho = 0.51$, p-value = 0.006 and $\rho = 0.56$, p-value = 0.002, respectively). This was also true for data from 2013 (see Appendix D, Table D.1).
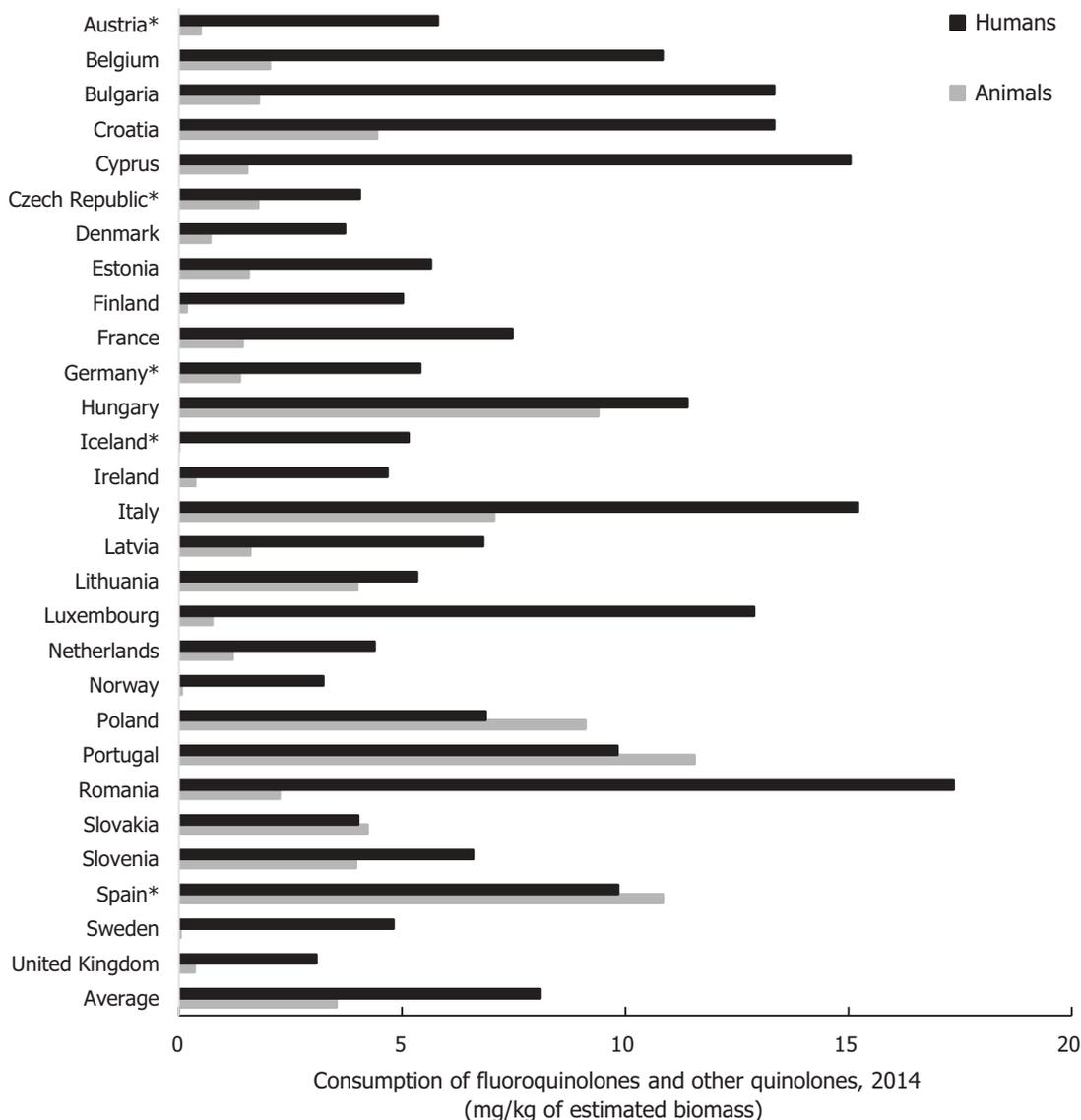
Asterisk (*) denotes that only community consumption was provided for human medicine. The population-weighted mean proportion (%) of the hospital sector from the 2014 total national consumption of antimicrobials for EU/EEA MSs that provided data for both sectors was 14.0%.

1) The estimates presented are crude and must be interpreted with caution. For limitations that hamper the comparison of consumption of antimicrobials in humans and animals, please see Section 14.

2) The average figure represents the population-weighted mean of data from included countries.

Figure 15: Population-corrected consumption of fluoroquinolones and other quinolones in humans and food-producing animals by country, EU/EEA MSs, 2014

7.2. Consumption of fluoroquinolones in humans and occurrence of resistance to fluoroquinolones in bacteria from humans

Quinolones consumed in humans are almost exclusively fluoroquinolones, which are used for the treatment of infections with both Gram-positive and Gram-negative bacteria, including *E. coli* infections and serious infections caused by *Salmonella* spp. and *Campylobacter* spp. Although fluoroquinolones are considered by the WHO as priority CIAs, they are widely used both at the hospital and in the community (WHO, 2017). AMEG has classified fluoroquinolones as belonging to their Category 2, which means that these antimicrobials should be used in veterinary medicine only when there are no alternative antimicrobials authorised for the respective target species and indication.

7.2.1. Invasive *Escherichia coli* isolates

In order to investigate the possible associations between the consumption of fluoroquinolones and the occurrence of resistance to fluoroquinolones in invasive *E. coli* isolates from humans, the total consumption of fluoroquinolones in humans, expressed in DDD per 1,000 inhabitants and per day, was analysed against the occurrence of fluoroquinolone resistance of invasive *E. coli* isolates from humans, for the years 2013, 2014 and 2015.

Data on the occurrence of resistance in invasive *E. coli* from humans were reported from all EU MSs and two EEA MSs in the period 2013–2015. Significantly-positive associations between resistance and total consumption were observed for *E. coli* for all three years ($p < 0.001$) (Table 14, Figure 16) where a higher total consumption correlated with a higher proportional increase in the occurrence of fluoroquinolone resistance. In 2013, each unit (per 1 DDD per 1,000 inhabitants and per day) increase in consumption of fluoroquinolones was associated with 56% higher probability of resistance in invasive *E. coli* isolates. The corresponding estimates for 2014 and 2015 were 48% and 53%, respectively (Table 14).

When analysing only community consumption data for fluoroquinolones against resistance to fluoroquinolones in invasive *E. coli* isolates from humans for the same period (2013–2015), strong associations ($p < 0.001$) were found for all three years.

Table 14: Results of logistic regression for total (community and hospital) consumption of fluoroquinolones in humans, expressed in DDD per 1,000 inhabitants and per day, and the probability of resistance to fluoroquinolones in invasive *E. coli* from humans, EU/EEA, 2013–2015 (see also Figure 16)

| Year	Countries included in the analysis	OR	95% PL CI	p-value
Invasive *E. coli*				
2013	AT, BE, BG, CY, CZ, DE, DK, EE, EL, ES, FI, FR, HR, HU, IE, IS, IT, LT, LU, LV, MT, NL, NO, PL, PT, RO, SE, SI, SK, UK (n = 30)	1.56	1.40–1.75	< 0.001
2014	AT, BE, BG, CY, CZ, DE, DK, EE, EL, ES, FI, FR, HR, HU, IE, IS, IT, LT, LU, LV, MT, NL, NO, PL, PT, RO, SE, SI, SK, UK (n = 30)	1.48	1.28–1.72	< 0.001
2015	AT, BE, BG, CY, CZ, DE, DK, EE, EL, ES, FI, FR, HR, HU, IE, IS, IT, LT, LU, LV, MT, NL, NO, PL, PT, RO, SE, SI, SK, UK (n = 30)	1.53	1.38–1.69	< 0.001

OR: odds ratio; PL CI: profile likelihood confidence interval, 95%.
OR varies from 0 to infinity. When OR equals 1 or CI includes 1, the association is not considered statistically-significant.
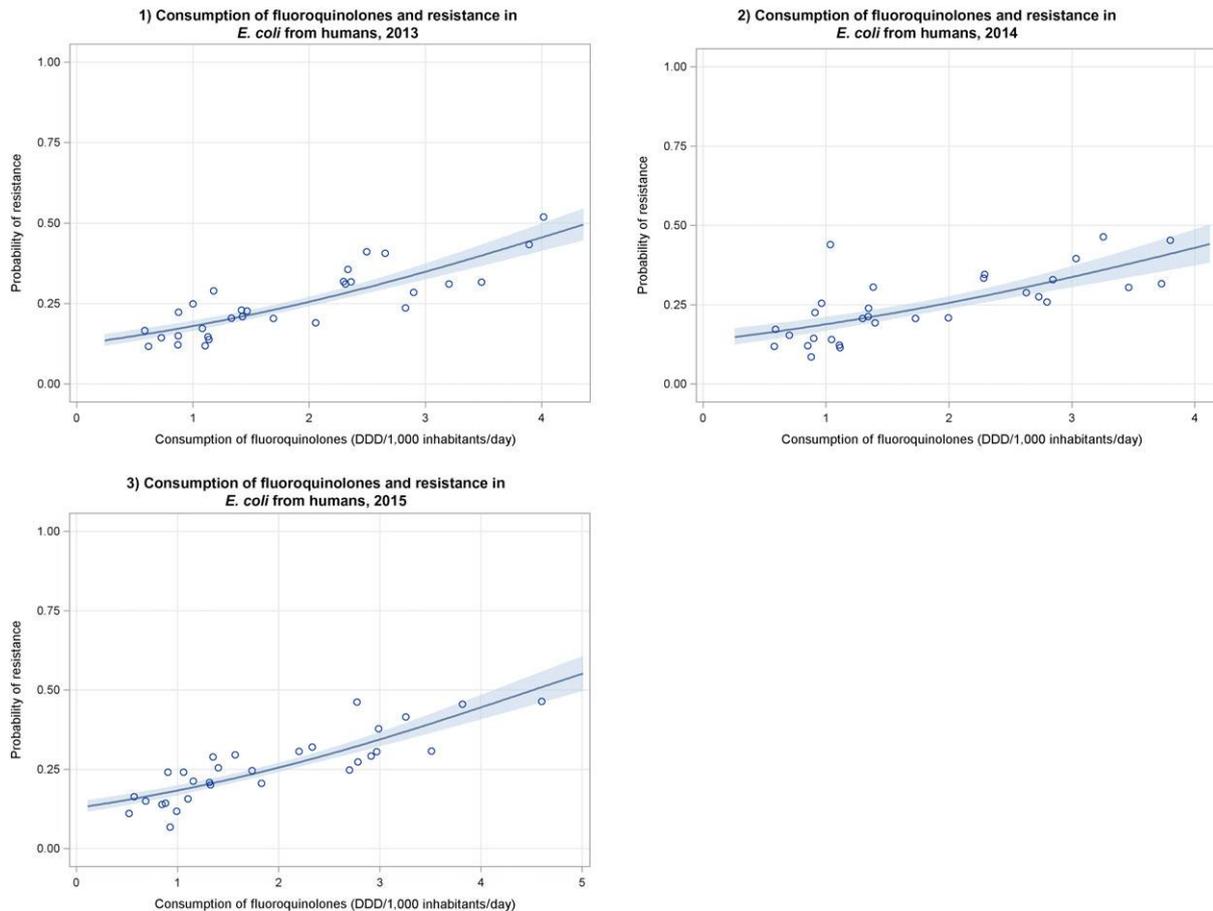
Dots represent countries included in the analysis.

Figure 16: Logistic regression analysis curves of the total (community and hospital) consumption of fluoroquinolones in humans, expressed in DDD per 1,000 inhabitants and per day, and the probability of resistance to fluoroquinolones in human invasive *E. coli*, EU/EEA, (1) 2013, (2) 2014 and (3) 2015 (see also Table 14)

7.2.2. *S.* Enteritidis, *S.* Typhimurium, monophasic *S.* Typhimurium and *S.* Infantis

The analysis of the total consumption (in the community and at the hospital) of fluoroquinolones and resistance of *S.* Enteritidis, *S.* Typhimurium and *S.* Infantis to fluoroquinolones showed no significant associations for the years 2013–2015. Only for monophasic *S.* Typhimurium in 2014, the total (community and hospital) consumption of fluoroquinolones was significantly associated with resistance to fluoroquinolones (Table 15, Figure 17). Data from only nine countries were included in this analysis. When omitting the country lying in the upper right corner of the graph (exhibiting large levels of both consumption and resistance) in the sensitivity analysis, the correlation between fluoroquinolone consumption and fluoroquinolone resistance in monophasic *S.* Typhimurium became insignificant (p = 0.353) (Table 15).

Table 15: Results of logistic regression for total (community and hospital) and for community consumption of fluoroquinolones in humans expressed in DDD per 1,000 inhabitants and per day and the probability of resistance to fluoroquinolones in selected salmonella serovars isolated from humans, EU/EEA, 2013–2015 (see also Figure 17)

Year	Countries included in the analysis	OR	95% PL CI	p-value
Monophasic *S.* Typhimurium – total consumption (community and hospital)				
2013	AT, DK, EL, ES, FR, HU, IE, IT, LU, NL (n = 10)	1.30	0.75–2.23	0.330
2014	AT, DK, ES, FR, IE, IT, LU, NL, PT (n = 9)	3.64	1.41–11.56	0.013
2015	AT, DK, EE, ES, FR, HU, IE, IT, LU, NL, PT (n = 11)	1.23	0.91–1.65	0.162
Monophasic *S.* Typhimurium – community consumption				
2013	AT, DK, EL, ES, FR, HU, IE, IT, LU, NL (n = 10)	1.33	0.73–2.44	0.349
2014	AT, DK, ES, FR, IE, IT, LU, NL, PT (n = 9)	4.17	1.32–18.3	0.028
2015	AT, DK, EE, ES, FR, HU, IE, IT, LU, NL, PT (n = 11)	1.25	0.91–1.75	0.184
***S.* Enteritidis – community consumption**				
2013	AT, BE, DE, EE, EL, ES, FI, FR, HU, IE, IT, LT, LU, LV, MT, NL, NO, RO, SI, SK, UK (n = 21)	1.18	0.79–1.77	0.412
2014	AT, BE, DE, ES, FI, FR, HU, IE, LT, LU, LV, MT, NL, NO, PT, RO, SI, SK, UK (n = 19)	1.74	1.01–3.06	0.047
2015	AT, DE, EE, EL, ES, FI, FR, HU, IE, IT, LT, LU, MT, NL, NO, PT, RO, SI, SK, UK (n = 20)	0.87	0.48–1.54	0.639

OR: odds ratio; PL CI: profile likelihood confidence interval, 95%.
OR varies from 0 to infinity. When OR equals 1 or CI includes 1, the association is not considered statistically-significant.
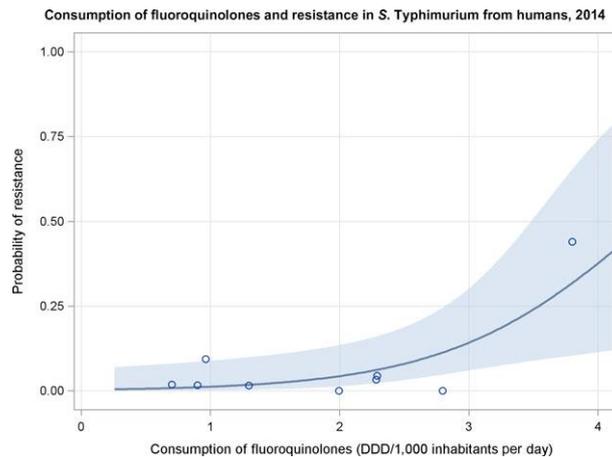
Dots represent countries included in the analysis.

Figure 17: Logistic regression analysis curves of the total (community and hospital) consumption of fluoroquinolones in humans, expressed in DDD per 1,000 inhabitants and per day, and the probability of resistance to fluoroquinolones in monophasic *S.* Typhimurium from humans, EU/EEA, 2014 (see also Table 15)

When only community consumption of fluoroquinolones was analysed against fluoroquinolone resistant isolates of *S.* Enteritidis and monophasic *S.* Typhimurium, significant correlations were observed for 2014. For *S.* Enteritidis, a higher consumption (by 1 unit DDD) of fluoroquinolones was associated with a 74% higher probability of resistance (Table 15) and for monophasic *S.* Typhimurium with a four-fold increased probability of resistance. When omitting the countries exhibiting high levels of consumption and resistance from the sensitivity analysis, the correlation between fluoroquinolones consumption and resistance in both *S.* Enteritidis and monophasic *S.* Typhimurium became insignificant (p = 0.454 and p = 0.834, respectively) (graphs not shown).

7.2.3. *Campylobacter jejuni* and *C. coli* from humans

Neither the total (community and hospital) consumption nor the community consumption (alone) of fluoroquinolones was associated with resistance to fluoroquinolones in *C. jejuni* and *C. coli* from humans for the years 2013, 2014 and 2015 (Table 16).

Table 16: Results of logistic regression for total (community and hospital) consumption of fluoroquinolones in humans, expressed in DDD per 1,000 inhabitants and per day, and the probability of resistance to fluoroquinolones in *C. jejuni* and *C. coli* isolates from humans, EU/EEA, 2013–2015

Year	Countries included in the analysis	OR	95% PL CI	p-value
C. jejuni				
2013	AT, DK, EE, ES, FR, IT, LT, LU, MT, NL, NO, RO, SI, SK, UK (n = 15)	1.36	0.95–1.98	0.095
2014	AT, EE, ES, FR, IT, LT, LU, MT, NL, NO, PT, RO, SI, SK (n = 14)	1.91	0.78–1.86	0.430
2015	AT, CY, DK, EE, ES, FI, FR, HU, IS, IT, LT, LU, MT, NL, NO, PT, RO, SI, SK, UK (n = 20)	1.33	0.94–1.93	0.114
C. coli				
2013	AT, ES, FR, IT, LT, LU, MT, NL, SI, SK, UK (n = 11)	1.29	0.84–2.01	0.251
2014	AT, ES, FR, LT, LU, MT, NL, PT, SI, SK (n = 10)	1.10	0.61–2.07	0.755
2015	AT, CY, EE, ES, FI, FR, IT, LT, LU, MT, NL, PT, RO, SI, SK, UK (n = 16)	1.10	0.70–1.81	0.671

OR: odds ratio; PL CI: profile likelihood confidence interval, 95%.
OR varies from 0 to infinity. When OR equals 1 or CI includes 1, the association is not considered statistically-significant.

7.3. Comparison of consumption of fluoroquinolones and other quinolones in animals with resistance to ciprofloxacin in bacteria from animals

7.3.1. In food-producing animals

In order to investigate possible relationships between the consumption of fluoroquinolones and other quinolones in animals and fluoroquinolones resistance in bacteria from food-producing animals, the SIMR to ciprofloxacin in indicator *E. coli*, *Salmonella* spp., *C. jejuni* and *C. coli* was compared with the consumption of fluoroquinolones and other quinolones in food-producing animals (expressed in mg per kg of estimated biomass) in 2013, and in 2014 and 2015 considered together, at the country level (Table 17). The category 'food-producing animals' includes broilers, pigs and cattle for 2013 and broilers, turkeys, pigs and calves for 2014–2015.

Marked variations in ciprofloxacin resistance in indicator *E. coli*, *Salmonella* spp., *C. jejuni* and *C. coli* were observed between countries involved in the analysis. Consumption of fluoroquinolones and other quinolones ranged between a few units and 10 mg per kg of estimated biomass. Statistically-significant positive associations between ciprofloxacin resistance in indicator *E. coli, Salmonella* spp., *C. jejuni* and *C. coli* and fluoroquinolones and other quinolones consumption in animals were observed in 2013, 2014 and 2015.

Regarding more specifically the relationship between consumption of all quinolones in food-producing animals and the risk of reduced susceptibility to ciprofloxacin assessed with the 2013 data (Table 17, Figure 18), a pattern was typically observed for *E. coli, C. jejuni, C. coli* and *Salmonella* spp. with two distinct groups of countries, with one group reporting low AMC and low AMR and the other group reporting high AMC and high AMR. Considering SIMR on *E. coli* from food-producing animals reported over 2014–2015 (Table 17, Figure 19), the inclusion of additional countries in the analysis, in particular those reporting intermediate amounts of consumption and levels of resistance, allowed a better assessment of the relationships between consumption and resistance, because a full range of values, including intermediate values, was available for the analysis.

Considering indicator *E. coli* and *Salmonella* spp. in 2014–2015, sensitivity analyses show that the association remain or become significantly positive after ignoring the three points shown on the upper middle area of the graph for indicator *E. coli* (23 observations, OR = 1.23, 95% PL CI: 1.13–1.31, p < 0.001) and the point displayed on the upper middle area of the graph for *Salmonella* spp. (7 observations; OR = 1.16, 95% PL CI: 1.03–1.32; p < 0.015).

Table 17: Results of logistic regression for consumption of fluoroquinolones and other quinolones in food-producing animals, expressed in mg/kg of estimated biomass/year, and probability of resistance to fluoroquinolones in bacteria from food-producing animals (see also Figures 18 and 19)

Year	Countries included in the analysis	OR[a]	95% PL CI	p-value
Indicator *E. coli*				
2013[b]	AT, BE, CH, DE, DK, ES, FI, HU, NL, PL, SE (n = 11)	1.22	1.13–1.32	< 0.001
2014–2015	AT, BE, BG, CY, CZ, DE, DK, EE, ES, FI, FR, HR, HU, IE, IT, LT, LV, NL, NO, PL, PT, RO, SE, SI, SK, UK (n = 26)	1.21	1.10–1.33	< 0.001
***Salmonella* spp.**				
2013[b]	BE, DE, DK, ES, IE, IT, NL, PL, UK (n = 9)	1.31	1.10–1.61	0.046
2014–2015	BE, CZ, DK, ES, FR, HR, IT, PT (n = 8)	1.17	0.99–1.41	0.072
C. jejuni				
2013[b]	AT, CH, DE, DK, ES, FI, NL, SE (n = 8)	1.25	1.12–1.42	< 0.001
C. coli				
2013[b]	CH, ES, FR, HU, NL, UK (n = 6)	1.26	1.09–1.51	0.002

OR: odds ratio; PL CI: profile likelihood confidence interval, 95%.
OR varies from 0 to infinity. When OR equals 1 or CI includes 1, the association is not considered statistically-significant.
Regarding resistance data, the category 'food-producing animals' includes broilers, pigs and cattle for 2013 and broilers, turkeys, pigs and calves for 2014–2015.
(a): OR estimated for 0.1-unit increment.
(b): In the absence of 2013 resistance data, proxy data for years prior to 2013 may have been used.

Dots represent the countries involved in the analysis. The category 'food-producing animals' includes cattle, broilers and pigs in 2013.

Figure 18: Logistic regression analysis curves of the consumption of fluoroquinolones and other quinolones in food-producing animals and the probability of resistance to ciprofloxacin in (1) indicator *E. coli* and (2) *Salmonella* spp. from food-producing animals, as well as in (3) *C. jejuni* from broilers and cattle and (4) *C. coli* from broilers and pigs, in 2013 (see also Table 17)
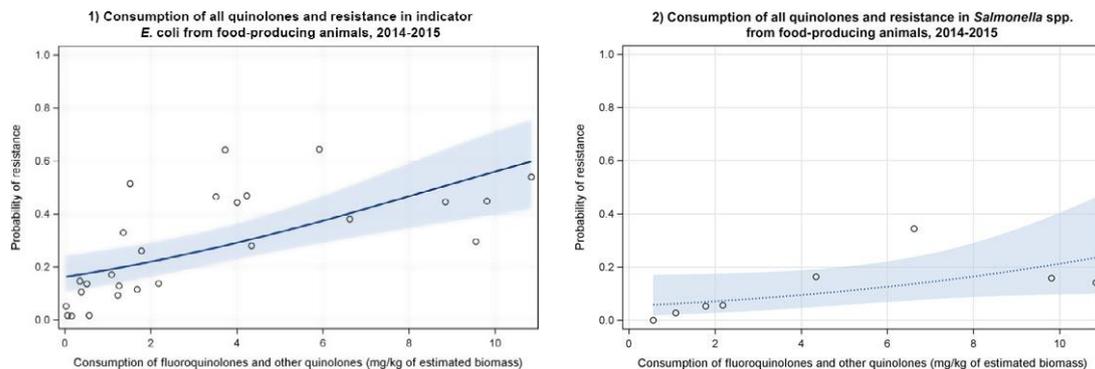
Dots represent the countries involved in the analysis. The category 'food-producing animals' includes broilers, turkeys, pigs and calves for 2014–2015.

Figure 19: Logistic regression analysis curves of consumption of fluoroquinolones and other quinolones in food-producing animals and the probability of resistance to ciprofloxacin in (1) indicator *E. coli* and (2) *Salmonella* spp. from food-producing animals for 2014–2015 (see also Table 17)

7.3.2. In pigs and in poultry

The estimated consumption of fluoroquinolones and other quinolones in pigs (expressed in DDDvet/kg of estimated biomass) was compared with the occurrence of resistance to ciprofloxacin in indicator *E. coli* isolates from slaughter pigs in years 2013 and 2015 for 12 and 18 reporting countries, respectively (Table 18). Both associations assessed for the years 2013 and 2015 were positive, although only the association for 2013 was statistically-significant. Considering indicator *E. coli* in 2015, sensitivity analysis shows that the association becomes significantly positive after ignoring the three points displayed in the upper left corner of the graph (15 observations, $OR_{0.1-unit}$ = 2.54, 95% PL CI: 1.99–3.25, p < 0.001) (Figure 20).

The estimated consumption of fluoroquinolones and other quinolones in poultry (expressed in DDDvet/kg of estimated biomass) was compared with the SIMR to ciprofloxacin in indicator *E. coli*, *Salmonella* spp. and *C. jejuni* from poultry (broilers and turkeys) in 2013 and 2014 (Table 18). Both data on ciprofloxacin resistance and consumption of fluoroquinolones and other quinolones in poultry were available together in 15 and 20 countries for *Salmonella* spp. in 2013 and 2014, respectively, and in 26 countries for indicator *E. coli* in 2014 and in 24 countries for *C. jejuni* in 2014. The associations assessed between the consumption of fluoroquinolones and other quinolones and resistance to cefotaxime in indicator *E. coli*, *Salmonella* spp. and *C. jejuni* in 2013 and 2015 were significantly positive, with the exception of the association for *Salmonella* spp. in 2013, which was not significant.

Table 18: Results of logistic regression for the estimated consumption of fluoroquinolones and other quinolones in pigs and poultry, expressed in DDDvet/kg of estimated biomass/year, and the probability of resistance to fluoroquinolones in bacteria from slaughter pigs and poultry (see also Figure 20)

Animal	Year	Countries included in the analysis	OR[a]	95% PL CI	p-value
Indicator *E. coli*					
Slaughter pigs	2013	AT, BE, DE, DK, ES, FI, FR, HU, NL, PL, SE, UK (n = 12)[b]	6.37	2.94–14.3	< 0.001
	2015	AT, BE, BG, CH, CY, DE, EE, FI, HR, HU, IE, LV, NO, PL, PT, RO, SE, SI (n = 18)	1.66	0.65–3.59	0.254
Poultry	2014	AT, BE, BG, CY, CZ, DE, DK, EE, ES, FI, FR, HR, HU, IE, IT, LT, LV, NL, NO, PL, PT, RO, SE, SI, SK, UK (n = 26)	1.92	1.28–3.31	< 0.001
***Salmonella* spp.**					
Poultry	2013	AT, BE, CZ, DE, DK, ES, FR, HU, IE, IT, NL, PL, SI, SK, UK (n = 15)[b]	1.31	0.98–1.85	0.021
	2014	AT, BE, BG, CY, CZ, DE, DK, ES, FR, HR, HU, IE, IS, IT, NL, PL, PT, RO, SI, SK, UK (n = 21)	1.49	1.09–2.19	0.048
C. jejuni					
Poultry	2014	AT, BE, CY, CZ, DE, DK, ES, FI, FR, HR, HU, IE, IS, IT, LT, LV, NL, PL, PT, RO, SE, SI, SK, UK (n = 24)	2.71	1.57–5.63	< 0.001

OR: odds ratio; PL CI: profile likelihood confidence interval, 95%.

OR varies from 0 to infinity. When OR equals 1 or CI includes 1, the association is not considered statistically-significant.

(a): OR estimated for 0.1-unit increment.

(b): In the absence of 2013 resistance data, proxy data for years prior to 2013 may have been used. Exceptions may occur when the over-dispersion correction proposed by Williams is used.
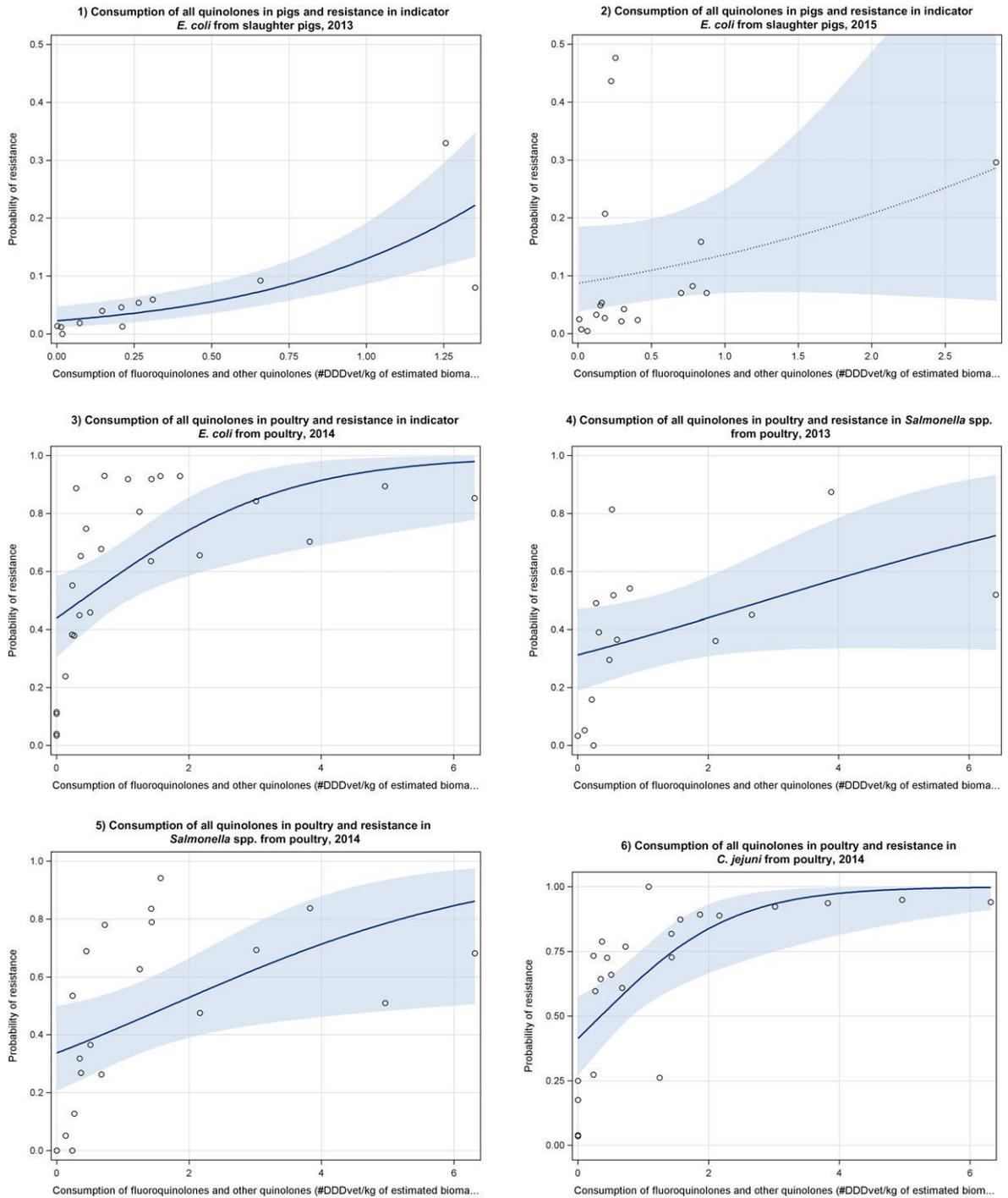
Dots represent the countries involved in the analysis. The category 'poultry' includes broilers for 2013 and broilers and turkeys for 2014. The scale used in graphs (5) and (6) is adapted according to the range of probabilities of resistance observed, in order to best show the distribution of data points. In graph (6), the dashed curve means that the corresponding association is not significant, although it becomes significant while disregarding the three outlying dots in the upper left hand corner of the graph.

Figure 20: Logistic regression analysis curves of the estimated consumption of all quinolones in pigs and the probability of resistance to ciprofloxacin in indicator *E. coli* from slaughter pigs in 2013 (1) and 2015 (2), and of the estimated consumption of all quinolones in poultry and the probability of resistance to ciprofloxacin in indicator *E. coli* from poultry in 2014 (3), in *Salmonella* spp. from poultry in 2013 (4) and 2014 (5) and in *Campylobacter jejuni* from poultry in 2014 (6) (see also Table 18)

7.4. Resistance to fluoroquinolones in bacteria from animals *versus* resistance in bacteria from humans

7.4.1. Resistance in invasive *E. coli* from humans and indicator *E. coli* from animals

Data on the occurrence of resistance to fluoroquinolones in invasive *E. coli* from humans (2013–2015) were compared to the occurrence of resistance to fluoroquinolones in indicator *E. coli* from pigs and cattle (2013 and 2015) as well as from fowl, broilers and turkeys (2013 and 2014). Significant correlations were found for all combinations analysed, with the exception of data for cattle for 2013 (Table 19). For 2013 for fowl and for 2014 for broilers or turkeys, an increase of resistance of *E. coli* by 1% was associated with an increase of probability of resistance in invasive *E. coli* from humans by 1%. In 2015, an increase of resistance of *E. coli* from cattle and pigs by 1% was associated with an increase of probability of resistance of invasive *E. coli* from humans by 4% and 2%, respectively (Table 19).

Table 19:   Results of logistic regression for the probability of resistance to fluoroquinolones in *E. coli* from food-producing animals and humans (see also Figures 21 and 22)

| Animal	Year	Countries	OR	95% PL CI	p-value
E. coli					
FPA[a]	2014–2015	AT, BE, BG, CY, CZ, DE, DK, EE, ES, FI, FR, HR, HU, IE, IT, LT, LV, NL, NO, PL, PT, RO, SE, SI, SK, UK (n = 26)	1.02	1.01–1.03	< 0.001
Broilers	2013	AT, BE, DE, DK, ES, FI, FR, HR, HU, NL, PL, SE (n = 12)	1.01	1.01–1.02	< 0.001
	2014	AT, BE, BG, CY, CZ, DE, DK, EE, EL, ES, FI, FR, HR, HU, IE, IT, LT, LV, MT, NL, NO, PL, PT, RO, SE, SI, SK, UK (n = 28)	1.01	1.01–1.02	0.001
Turkeys	2014	AT, DE, ES, FR, HU, IT, PL, PT, RO, SE, UK (n = 11)	1.01	1.01–1.02	< 0.001
Pigs	2013	AT, BE, DE, DK, ES, FI, FR, HU, NL, PL, SE, UK (n = 12)	1.04	1.02–1.05	< 0.001
	2015	AT, BE, BG, CY, CZ, DE, DK, EE, EL, ES, FI, FR, HR, HU, IE, IT, LT, LV, MT, NL, NO, PL, PT, RO, SE, SI, SK, UK (n = 28)	1.02	1.01–1.03	0.001

FPA: food-producing animals; OR: odds ratio; PL CI: profile likelihood confidence interval, 95%.
Note: OR varies from 0 to infinity. When OR equals 1 or CI includes 1, the association is not considered statistically-significant.
(a): Food-producing animals: for 2014–2015 this category includes broilers, turkeys, pigs and calves.
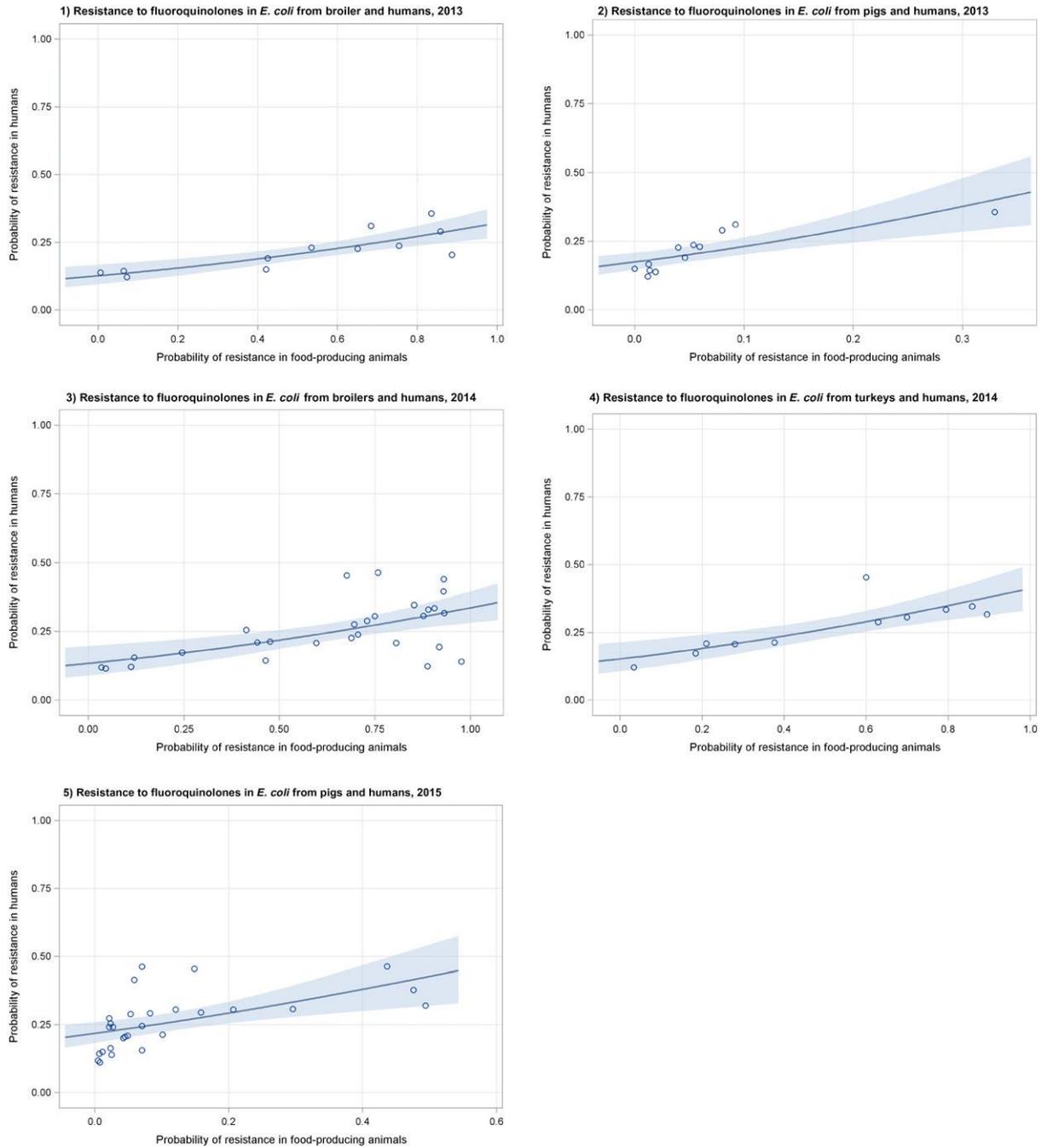
Dots represent countries included in the analysis.

Figure 21: Logistic regression analysis curves of the probability of resistance to fluoroquinolones in *E. coli* from food-producing animals and humans, 2013–2015 (see also Table 19)

Using aggregated data for 2014 and 2015, resistance to fluoroquinolones of invasive *E. coli* from humans significantly correlated with resistance to fluoroquinolones in indicator *E. coli* from food-producing animals (SIMR) (Figure 22).

Resistance to fluoroquinolones in *E. coli* from food-producing animals and humans, 2014–2015
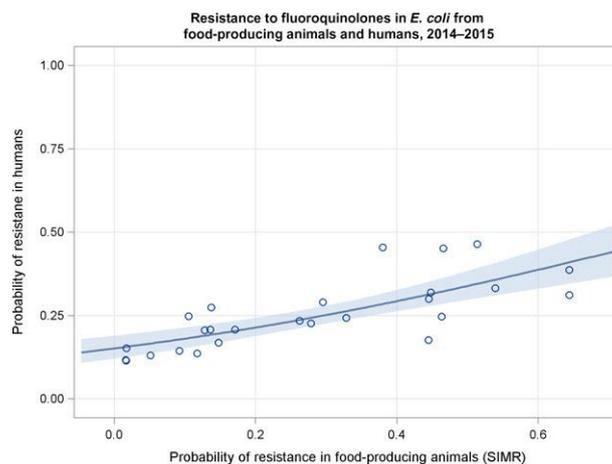
Dots represent countries included in the analysis.

Figure 22: Logistic regression analysis curves of the probability of resistance to fluoroquinolones in invasive *E. coli* from humans and the probability of resistance in indicator *E. coli* (SIMR) from food-producing animals (combined data for 2014–2015) (see also Table 19)

This correlation between resistance to fluoroquinolones of *E. coli* from humans and food-producing animals remained significant even when two countries (outliers) were excluded from the analysis.

7.4.2. Resistance in *S.* Typhimurium, *S.* Enteritidis and *S.* Infantis from humans and animals

For *S.* Typhimurium, *S.* Enteritidis and *S.* Infantis, few countries reported data from both cases of human infection and from broilers, fowl, pigs or cattle in the period 2013–2015. When at least five countries reported adequate data, analysis showed significant correlation only for *S.* Infantis from broilers and from humans in 2013 but no other significant correlations with the other combinations (Table 20, Figure 23).

Table 20: Results of logistic regression for probability of resistance to fluoroquinolones in *Salmonella* spp. and selected serovars from food-producing animals and humans (see also Figure 23)

Animal	Year	Countries	OR	95% PL CI	p-value
***Salmonella* spp.**					
Food-producing animals	2014–2015	BE, DK, ES, FR, IT, PT (n = 6)	1.01	0.97–1.06	0.558
***S.* Typhimurium**					
Broilers	2014	BE, DE, DK, ES, FR, PT (n = 6)	0.97	0.85–1.03	0.476
***S.* Enteritidis**					
Broilers	2013[a]	AT, BE, DE, ES, FR, HU, IT, NL, RO, SK (n = 10)	1.02	0.99–1.05	0.066
	2014	AT, BE, HU, NL, RO (n = 5)	1.01	0.99–1.03	0.297
***S.* Infantis**					
Broilers	2013[a]	AT, BE, DE, ES, IT, NL, RO, SI, SK (n = 9)	1.04	1.01–1.08	0.024
	2014	AT, BE, DE, HU, IT, NL, SI, SK (n = 8)	1.04	0.99–1.09	0.091

OR: odds ratio; PL CI: profile likelihood confidence interval, 95%.
OR varies from 0 to infinity. When OR equals 1 or the CI includes 1, the association is not considered statistically-significant.
The category 'food-producing animals' includes broilers, turkeys, pigs and calves for 2014–2015.
(a): In the absence of 2013 resistance data, proxy data for years prior to 2013 may have been used.

Resistance to fluoroquinolones in *S.* Infantis
from food-producing animals and humans, 2013
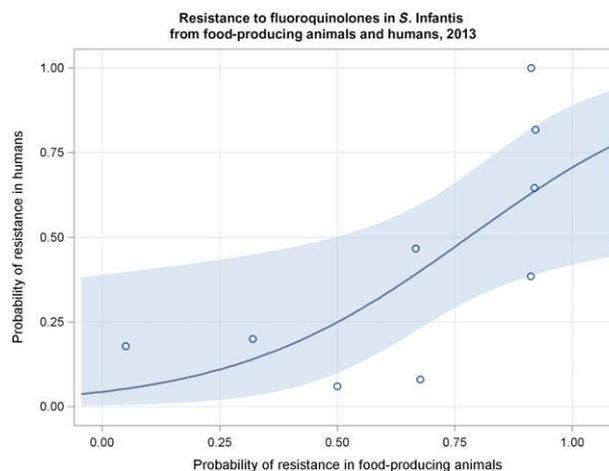
Dots represent countries included in the analysis.

Figure 23: Logistic regression analysis curves of the probability of resistance to fluoroquinolones in *S.* Infantis from food-producing animals and humans, 2013 (see also Table 20)

Combining data from 2014 and 2015 and comparing resistance of *Salmonella* spp. to fluoroquinolones from humans and from food-producing animals (using the SIMR), no significant correlations were found.

7.4.3.   Resistance in *C. jejuni* and *C. coli* from animals and humans

In 2013, resistance of *C. jejuni* to fluoroquinolones from broilers was significantly correlated to resistance of *C. jejuni* to fluoroquinolones from humans (Table 21, Figure 24). Similarly, in 2014, significant correlations were found between resistance of *C. jejuni* to fluoroquinolones from broilers (but not from turkeys where the number of countries available for analysis was lower) and from humans (Table 21, Figure 24).

Table 21:   Results of logistic regression for probability of resistance to fluoroquinolones in *C. jejuni* and in *C. coli* from food-producing animals and humans, EU/EEA, 2013–2014 (see also Figures 24 and 25)

Animal	Year	Countries	OR	95% PL CI	p-value
C. jejuni					
Broilers	2013	AT, DK, ES, FR, NL, NO, SI, UK (n = 8)	1.03	1.02–1.04	< 0.001
	2014	AT, ES, FR, IT, LT, NL, PT, RO, SI, SK (n = 10)	1.06	1.03–1.09	< 0.001
Turkeys	2014	AT, ES, FR, IT, PT, RO (n = 6)	1.04	0.99–1.09	0.160
C. coli					
Broilers	2013	AT, ES, FR, NL, UK (n = 5)	1.05	1.03–1.06	< 0.001

OR: odds ratio; PL CI: profile likelihood confidence interval, 95%.
Note: OR varies from 0 to infinity. When OR equals 1 or CI includes 1, the association is not considered statistically-significant.

1) Resistance to fluoroquinolones in *C. jejuni* from broilers and humans, 2013

2) Resistance to fluoroquinolones in *C. jejuni* from broilers and humans, 2014
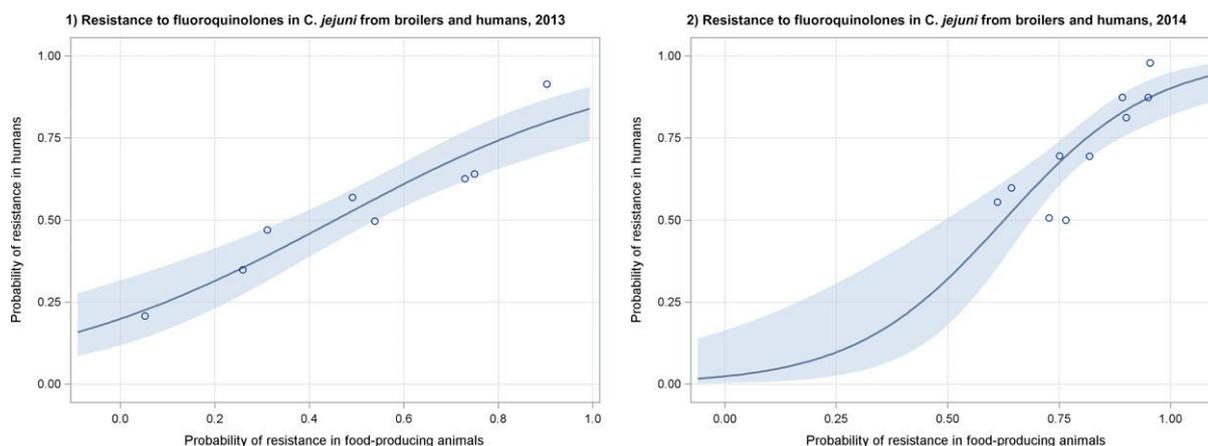
Dots represent countries included in the analysis.

Figure 24: Logistic regression analysis curves of the probability of resistance to fluoroquinolones in *Campylobacter jejuni* from food-producing animals and humans, (1) 2013 and (2) 2014 (see also Table 21)

Resistance of *C. coli* to fluoroquinolones from broilers was significantly correlated to resistance of *C. coli* to fluoroquinolones from humans in 2013 (Table 21, Figure 25). Data on *C. coli* from pigs was only available from four countries from both sectors for 2013–2015 and were therefore not included.

Resistance to fluoroquinolones in *C. coli* from broilers and humans, 2013
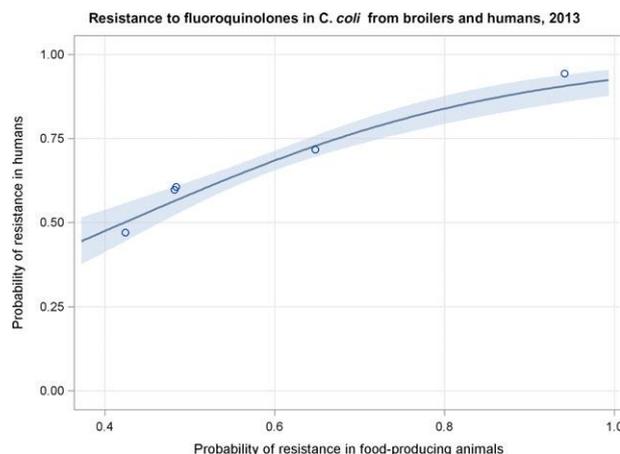
Dots represent countries included in the analysis.

Figure 25: Logistic regression analysis curves of the probability of resistance to fluoroquinolones in *Campylobacter coli* from broilers and humans, 2013 (see also Table 21)

7.5. Consumption of fluoroquinolones in food-producing animals *versus* resistance in bacteria from humans

In order to investigate possible relationships between the consumption of fluoroquinolones or other quinolones in food-producing animals and fluoroquinolone resistance in bacteria causing infections in humans, the occurrence of resistance in *E. coli* BSI and *Salmonella* spp. from humans was compared with the total consumption in food-producing animals of fluoroquinolones and quinolones (milligrams per kilogram of estimated biomass) in 2013, 2014 and 2015 at the country level (Table 22).

Significant positive associations between fluoroquinolone resistance in *E. coli* BSI from humans and the total consumption in animals (fluoroquinolones and other quinolones) were observed in 2013, 2014 and 2015. The assessed strength of the association is remarkably consistent over the years studied; a higher consumption of 1 mg/kg of estimated biomass of fluoroquinolones and other quinolones resulting in an increase of the risk of resistance to fluoroquinolones in *E. coli* BSI in humans of around 10%.

Regarding *Salmonella* spp. isolates, although the estimated strength of the positive association is also rather stable over the years, the association is only significant for the year 2014.

Table 22: Results of logistic regression for consumption of fluoroquinolones and other quinolones in food-producing animals, expressed in mg/kg of estimated biomass/year, and probability of resistance to fluoroquinolones in bacteria causing infections in humans

Year	Countries included in the analysis	OR	95% PL CI	p-value
E. coli				
2013	AT, BE, BG, CY, CZ, DE, DK, EE, ES, FI, FR, HU, IE, IS, IT, LT, LU, LV, NL, NO, PL, PT, SE, SI, SK, UK (n = 26)	1.10	1.04–1.12	0.001
2014	AT, BE, BG, CY, CZ, DE, DK, EE, ES, FI, FR, HR, HU, IE, IS, IT, LT, LU, LV, NL, NO, PL, PT, RO, SE, SI, SK, UK (n = 28)	1.09	1.03–1.15	0.002
2015	AT, BE, BG, CY, DE, DK, EE, FI, FR, HR, HU, IE, IS, IT, LV, NL, NO, PL, PT, RO, SE, SI, UK (n = 23)	1.11	1.05–1.18	0.001
Salmonella spp.				
2013	AT, BE, DE, DK, EE, ES, FI, FR, HU, IE, IS, IT, LT, LU, LV, NL, NO, SI, SK, UK (n = 20)	1.08	0.99–1.17	0.082
2014	AT, BE, DE, DK, EE, ES, FI, FR, HU, IE, IT, LT, LU, LV, NL, NO, PT, RO, SI, SK, UK (n = 21)	1.09	1.01–1.17	0.027
2015	AT, DE, DK, EE, FI, FR, HU, IE, IS, IT, NL, NO, PT, RO, SI, UK (n = 16)	1.08	0.99–1.18	0.085

OR: odds ratio; PL CI: profile likelihood confidence interval.
OR varies from 0 to infinity. When OR equals 1 or CI includes 1, the association is not considered statistically-significant.

7.6. Multivariate analysis

7.6.1. *Escherichia coli*

As reported in the univariate analysis, in both humans and animals, the consumption of fluoroquinolones and other quinolones was highly significantly related to resistance in invasive *E. coli* and to resistance in commensal *E. coli*, respectively (Figure 26). According to the R^2, 69% of the variance of resistance is explained by the corresponding latent variable: fluoroquinolones and other quinolones consumption in humans. Only 49% of the variance of resistance in animals is explained by the corresponding latent variable fluoroquinolones and other quinolones consumption in animals. The mean redundancy values, reflecting the prediction ability of the models, indicate that 69% and 38% (not shown in the figure below) of the variability of resistance indicators are, respectively, explained in humans and in animals.

The strong relationship (path coefficient = 0.83 and $p = 1.6 \times 10^{-7}$) between fluoroquinolones and other quinolones consumption in humans and resistance in invasive *E. coli* might explain the dropping of the path between resistance in animals and resistance in humans, this relationship being non-significant (p = 0.08).
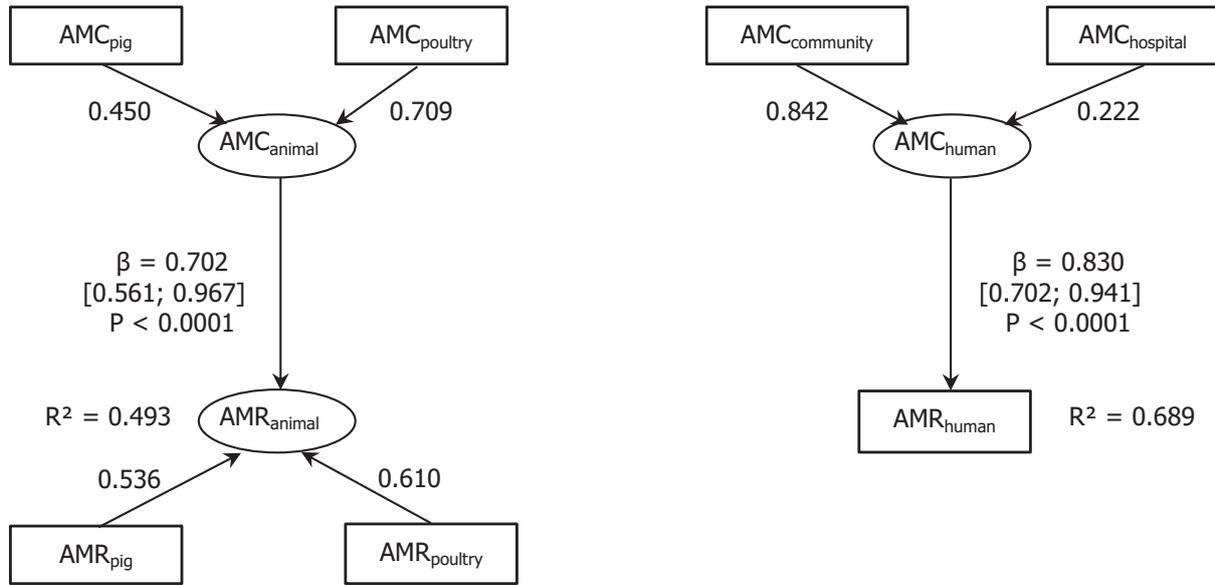
26 countries: AT*, BE, BG, CY, CZ*[†], DE*, DK, EE, ES*[†], FI, FR, HR, HU, IE, IT, LT[†], LV, NL, NO, PL, PT, RO, SE, SI, SK[†], UK (Goodness-of-fit = 0.668).
[†]For these countries, the estimated consumption in pigs in 2014 was used as a proxy for 2015 missing data.
*For these countries, consumption in hospital was estimated.

Figure 26: Diagram of the PLS-PM of resistance to fluoroquinolones in human invasive *E. coli* (2014 and 2015) considering resistance to fluoroquinolones in indicator *E. coli* from animals (pigs 2015 and poultry 2014), consumption of fluoroquinolones and other quinolones in humans (2014–2015 average, expressed in DDD per 1,000 inhabitants and per day), in animals (pigs in 2015 and poultry in 2014, expressed in DDDvet/kg of estimated biomass)

7.6.2. *Salmonella* spp.

Multivariate analysis involved only 10 countries for which all necessary data were available. This limited sample hindered the proper estimation of confidence intervals by the bootstrapping method.

The PLS-PM model (Figure 27) showed that the direct effect of resistance in animals (poultry and pigs) on resistance in humans was estimated at 0.754 whereas the indirect effect of fluoroquinolones and other quinolones consumption in animals (poultry and pigs) was assessed at 0.585 (not shown in the figure below). According to R^2, 60% of the variance of the resistance in animals is explained by the corresponding latent variable 'fluoroquinolones and other quinolones consumption in animals', whereas 57% of the variance of the resistance in humans is explained by the latent variable 'fluoroquinolones resistance in animals (poultry and pigs). Fluoroquinolones and other quinolones consumption in humans was a non-significant variable.
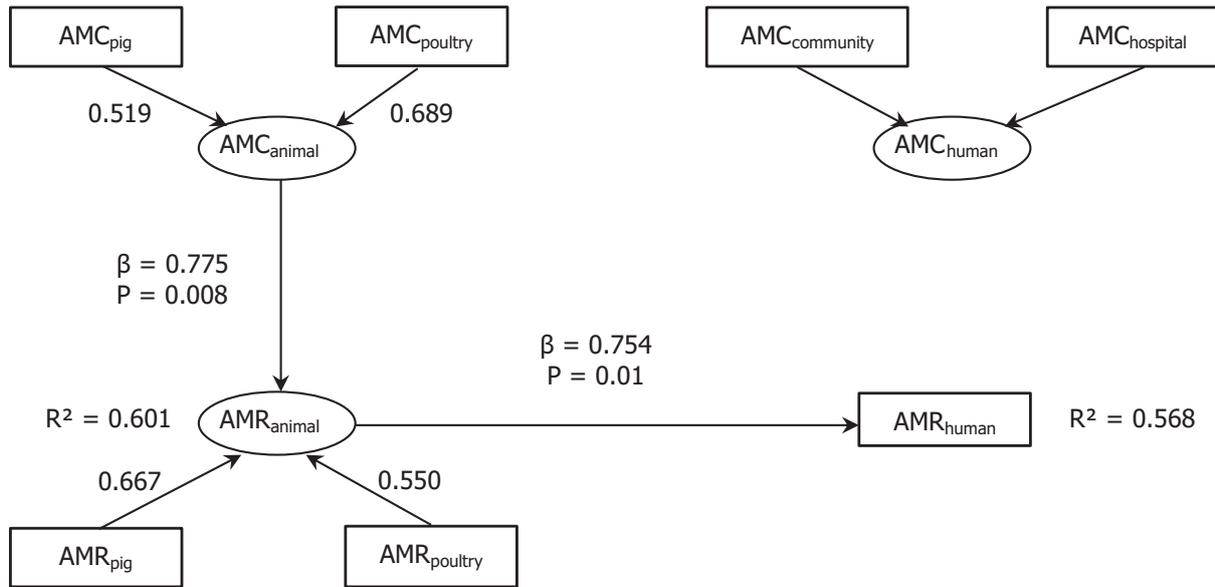
10 countries involved: BE, DE*, DK, ES*[†], FR, HU, PT, RO, SK[†], UK (Goodness-of-fit = 0.627).
[†]For these countries, the estimated consumption in pigs in 2014 was used as a proxy for 2015 missing data.
*For these countries, consumption in hospital was estimated.

Figure 27: Diagram of the PLS-PM model of resistance to fluoroquinolones in *Salmonella* spp. from humans (in 2014 and 2015) considering resistance to fluoroquinolones in *Salmonella* spp. from animals (in pigs in 2015 and in poultry in 2014), consumption of fluoroquinolones and other quinolones in humans (average 2014–2015, expressed in DDD per 1,000 inhabitants and per day), in animals (in pigs in 2015 and in poultry in 2014, expressed in DDDvet/kg of estimated biomass)

7.6.3. *Campylobacter jejuni*

Considering the few data on resistance in *Campylobacter jejuni* from pigs available, the model only included data on resistance in *C. jejuni* from poultry, so that 15 countries were involved in the model (Figure 28).

According to R^2, 73% of the variance of resistance in humans is explained by resistance in animals, where variance is conversely poorly explained by consumption of fluoroquinolones and quinolones ($R^2 = 0.43$). The pathways are similar to those observed in the model on *Salmonella* spp., such as the path effects estimations. The 'direct effect' of resistance in animals (poultry) on resistance in humans was estimated at 0.853, and the 'indirect effect' of fluoroquinolones and other quinolones consumption in animals (poultry and pigs) was assessed at 0.559. Fluoroquinolones and other quinolones consumption in humans was a non-significant latent variable in the model.
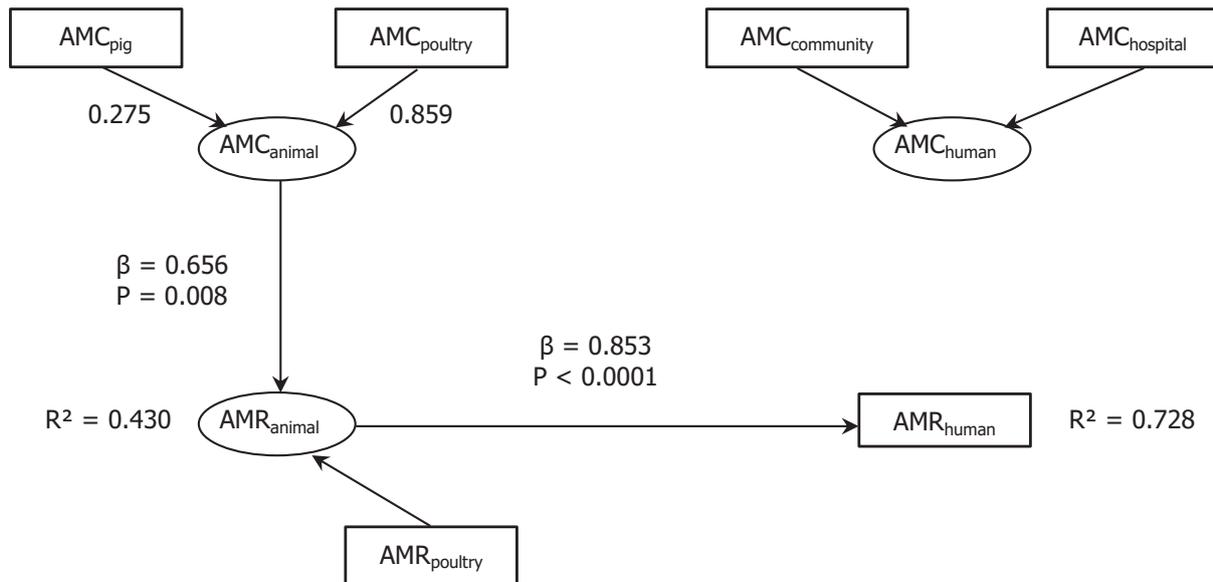
15 countries: AT*, CY, DK, ES*, FI, FR, IS, IT, LT, NL, PT, RO, SI, SK, UK (Goodness-of-fit = 0.617).
*For these countries, consumption in hospital was estimated.

Figure 28: Diagram of the PLS-PM model of resistance to fluoroquinolones in *Campylobacter jejuni* in humans (in 2014) considering resistance to fluoroquinolones in *Campylobacter jejuni* from animals (poultry in 2014), consumption of fluoroquinolones and other quinolones in humans (expressed in DDD per 1,000 inhabitants and per day in 2014 and 2015), in animals (poultry and pigs, 2014, expressed in DDDvet/kg of estimated biomass)

7.7. Key findings on fluoroquinolones and other quinolones

- Fluoroquinolones are categorised by AMEG in category 2, implying that restrictions in use in food-producing animals are needed (EMA/AMEG, 2016). Overall, in most countries, the consumption of fluoroquinolones was lower in food-producing animals than in humans, but the difference in consumption was less marked than that of the 3rd- and 4th-generation cephalosporins. There was a significant correlation between consumption in humans and animals at the country level. In human medicine, fluoroquinolones are mostly consumed in the community, in contrast with the 3rd- and 4th-generation cephalosporins, which are primarily consumed at the hospital.

- In humans, significant positive associations between consumption of and resistance to fluoroquinolones were only observed for invasive *E. coli*, but not for *Salmonella* spp. or *Campylobacter* spp. As almost all countries reported data, it seems reasonable to conclude that the consumption of fluoroquinolones, especially in the community, contributes to resistance to fluoroquinolones in invasive *E. coli* from humans at the EU/EEA level.

- In food-producing animals (considering the SIMR), and also specifically in pigs and poultry, significant positive associations between consumption of and resistance of indicator *E. coli*, *Salmonella* and *Campylobacter* spp. to fluoroquinolones were generally observed over the period of study.

- Comparing (microbiological) resistance to fluoroquinolones in invasive *E. coli* from humans and animals, significant positive associations were found for all combinations analysed. In addition, the SIMR to fluoroquinolones in indicator *E. coli* from food-producing animals for 2014–2015 was also significantly associated with resistance in invasive *E. coli* from humans.

- For resistance to fluoroquinolones in *S.* Typhimurium, *S.* Enteritidis and *S.* Infantis from animals and humans, data were sparse. Sufficient data for analysis were only available for the interaction between broilers and humans. No significant association was found with the only exception being *S.* Infantis from broilers and humans. In addition, analysing aggregate data from two years (2014 and 2015) and from all animal species (SIMR) no correlation was found

between resistance to fluoroquinolones of *Salmonella* spp. from humans and food-producing animals.

- Strong significant positive associations were found between resistance to fluoroquinolones in *C. jejuni* and *C. coli* from humans and from broilers.
- In the multivariate analysis on *E. coli*, the only significant effect on resistance to fluoroquinolones in invasive *E. coli* from humans was the strong direct impact of the consumption of fluoroquinolones in humans. This multivariate analysis also showed that consumption of fluoroquinolones in animals had a strong impact on resistance in bacteria from animals. No significant association was observed between resistance in indicator *E. coli* from food-producing animals and resistance in invasive *E. coli* from humans.
- In both multivariate analyses on *Salmonella* spp. and *C. jejuni*, resistance to fluoroquinolones and other quinolones in bacteria from humans was significantly related to resistance in bacteria from food-producing animals, which, in turn, was significantly linked to the consumption of fluoroquinolones and other quinolones in such animals.

8.   Polymyxins

8.1.   Consumption of polymyxins by country

Polymyxins are regarded by the WHO as CIAs of the highest priority (5th revision—WHO list of critically important antimicrobials (CIA)) (WHO, 2017)). AMEG has recently re-classified polymyxins as belonging to their Category 2 (EMA/AMEG, 2016). In 2014, the population-weighted mean consumption of polymyxins in humans and food-producing animals was 0.03 and 10.0 mg per kg of estimated biomass, respectively. The corresponding ranges were 0–0.1 (median 0.01) and 0–36.1 (median 1.3) mg per kg, respectively. Population-corrected consumption of polymyxins in humans and food-producing animals by country is shown in Figure 29A. Because of the large differences between consumption in humans and in animals, consumption in humans is illustrated separately in Figure 29B with a different scale.

Overall, the consumption of polymyxins in food-producing animals by far outweighed that reported in humans but there was no consumption in food-producing animals in Finland, Iceland and Norway and lower consumption in animals than in humans in one country. Also, the variation between countries in the quantities of polymyxins consumed in food-producing animals was very wide. There was no significant correlation within country between consumption of polymyxins in humans and food-producing animals (Spearman's rank correlation, $\rho = 0.03$).
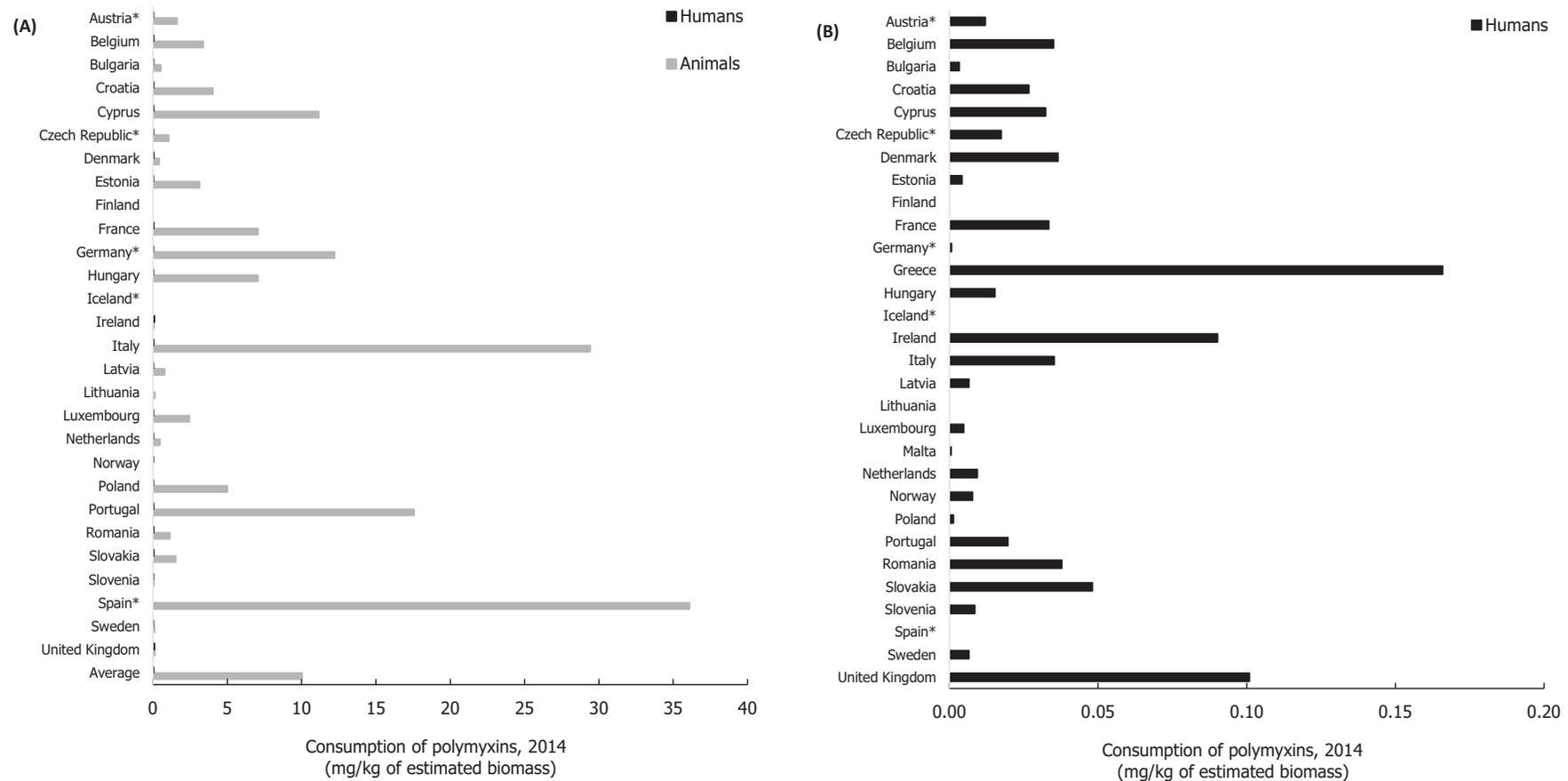
Asterisk (*) denotes that only community consumption was provided for human medicine. The population-weighted mean proportion (%) of the hospital sector from the 2014 total national consumption of antimicrobials for EU/EEA MSs that provided data for both sectors is 51.4%.

1) The estimates presented are crude and must be interpreted with caution. For limitations that hamper the comparison of consumption of antimicrobials by humans and animals, please see Section 14.

2) The average figure represents the population-weighted mean of data from included countries.

Figure 29: Population-corrected consumption of polymyxins in humans and food-producing animals by country in 28 EU/EEA MSs (A) and in humans only in 30 countries (B) in 2014

8.2. Consumption of polymyxins in humans *versus* resistance to polymyxins in bacteria from humans

Data on the occurrence of resistance to polymyxins in invasive *Klebsiella pneumoniae* from humans were correlated with consumption of polymyxins from all EU countries and two EEA countries in the period 2013–2015. Strong correlations between resistance and total (community and hospital) consumption were observed for *K. pneumoniae* for 2014 and 2015 ($p < 0.05$) (Table 23, Figure 30). Higher total consumption correlated with a higher occurrence of polymyxin resistance. A higher consumption (by 1 unit DDD) of polymyxin was associated with a higher probability of resistance in invasive *K. pneumoniae* isolates (17% in 2014 and 13% in 2015) (Table 23).

Table 23: Results of logistic regression for total (community and hospital) consumption and for community and hospital consumption of polymyxins in humans expressed in DDD per 1,000 inhabitants and per day and the probability of resistance to polymyxins in invasive *K. pneumoniae* from humans, EU/EEA, 2013–2015 (see also Figures 30 and 31)

Year	Countries included in the analysis	OR	95% PL CI	p-value
K. pneumoniae – total consumption (community and hospitals)				
2013	BE, CY, CZ, DE, EL, FR, HU, IT, LU, MT, NL, PL, PT, RO, SK, UK (n = 16)	1.13	0.96–1.31	0.112
2014	BE, CY, CZ, DE, EL, FR, HU, IT, MT, NL, PL, PT, RO, SK, UK (n = 15)	1.17	1.01–1.34	0.028
2015	AT, BE, CY, CZ, DE, EL, ES, FR, HU, IT, MT, NL, PL, PT, RO, SK, UK (n = 17)	1.13	1.02–1.26	0.014
K. pneumoniae – community consumption				
2013	BE, CY, CZ, DE, EL, FR, HU, IT, LU, MT, NL, PL, PT, RO, SK, UK (n = 16)	1.01	0.65–1.37	0.953
2014	BE, CY, CZ, DE, EL, FR, HU, IT, MT, NL, PL, PT, RO, SK, UK (n = 15)	1.19	0.84–1.58	0.259
2015	AT, BE, CY, CZ, DE, EL, ES, FR, HU, IT, MT, NL, PL, PT, RO, SK, UK (n = 17)	1.06	0.82–1.31	0.599
K. pneumoniae – hospital consumption				
2013	BE, CY, EL, FR, HU, IT, LU, MT, NL, PT, RO, SK, UK (n = 13)	1.21	0.97–1.46	0.062
2014	BE, CY, EL, FR, HU, IT, MT, NL, NO, PL, PT, RO, SK, UK (n = 14)	1.17	0.96–1.39	0.085
2015	BE, CY, EL, FR, HU, IT, MT, NL, NO, PL, PT, RO, SK, UK (n = 14)	1.22	1.07–1.38	0.001

OR: odds ratio; PL CI: profile likelihood confidence interval, 95%.
OR varies from 0 to infinity. When OR equals 1 or CI includes 1, the association is not considered statistically-significant.
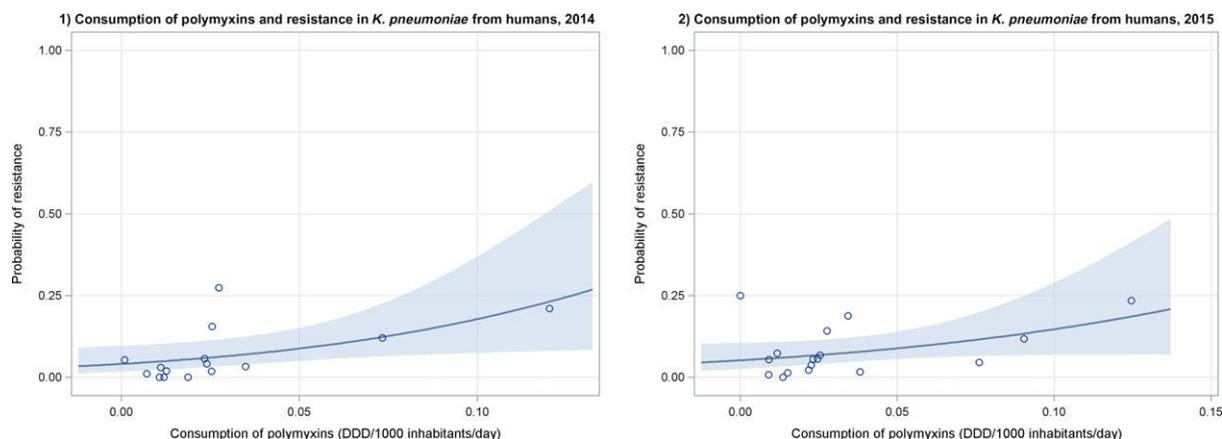
Dots represent countries included in the analysis.

Figure 30: Logistic regression analysis curves of the total (community and hospital) consumption of polymyxins in humans expressed in DDD per 1,000 inhabitants and per day, and the probability of resistance to polymyxins in invasive *K. pneumoniae* from humans, EU/EEA, (1) 2014 and (2) 2015 (see also Table 23)

When analysing only community consumption data for polymyxins against resistance to polymyxins in invasive *K. pneumoniae* isolates from humans for the same period (2013–2015), no significant correlations were found for all three years.

When analysing only hospital consumption data for polymyxins against resistance to polymyxins in invasive *K. pneumoniae* isolates from humans for the same period (2013–2015), strong correlations (p = 0.001) were found only for 2015 (Figure 31).

Consumption of polymyxins in hospitals and resistance in
K. pneumoniae from humans, 2015
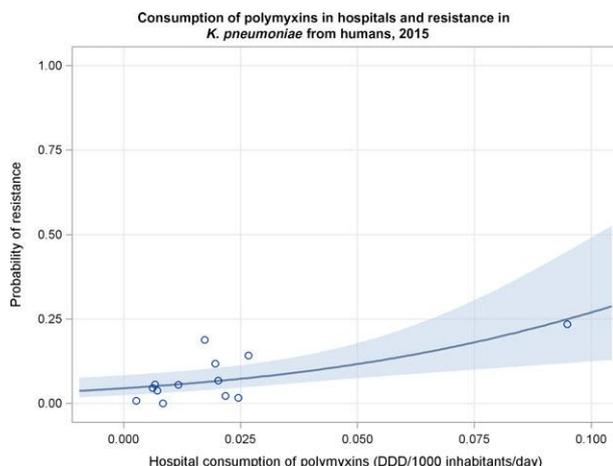
Figure 31: Logistic regression analysis curves of hospital consumption of polymyxins in humans expressed in DDD per 1,000 inhabitants and per day, and the probability of resistance to polymyxins in invasive *K. pneumoniae* from humans, EU/EEA, 2015 (see also Table 23)

8.3.   Comparison of consumption of polymyxins in animals with resistance to colistin in bacteria from animals

Susceptibility testing to colistin in bacteria from food-producing animals commenced on a mandatory basis in the EU MSs in 2014. Data on resistance to colistin data were therefore not available for the year 2013. In addition, susceptibility to colistin was only addressed for indicator *E. coli*, as ECOFFs for colistin in salmonella serovars are still awaiting determination by EUCAST.

8.3.1.   In food-producing animals

In order to investigate possible relationships between the consumption of polymyxins and colistin resistance, the SIMR to colistin in indicator *E. coli* from food-producing animals was compared with the consumption of polymyxins in animals (expressed in mg per kg of estimated biomass) for 2014–2015 (average consumption over 2014–2015) at the country level (Table 24, Figure 32). The category 'food-producing animals' includes broilers, turkeys, pigs and veal calves for 2014–2015. Colistin resistance observed in indicator *E. coli* from food-producing animals was typically low in the countries included in the analysis. Consumption of polymyxins ranged between a few units and up to nearly 40 mg per kg of estimated biomass. Statistically-significant positive associations between colistin resistance in indicator *E. coli* and polymyxin consumption in animals were observed in 2014–2015.

Table 24: Results of logistic regression for consumption of polymyxins in food-producing animals, expressed in mg/kg of estimated biomass/year, and probability of resistance to polymyxins in indicator *E. coli* from food-producing animals (see also Figure 32)

Year	Countries included in the analysis	OR	95% PL CI	p-value
Indicator *E. coli*				
2014–2015	AT, BE, BG, CY, CZ, DE, DK, EE, ES, FI, FR, HR, HU, IE, IT, LT, LV, NL, NO, PL, PT, RO, SE, SI, SK, UK (n = 26)	1.078	1.04–1.11	< 0.001

OR: odds ratio; PL CI: profile likelihood confidence interval, 95%.
Note: OR varies from 0 to infinity. When OR equals 1 or CI includes 1, the association is not considered statistically-significant.
Regarding resistance data, the category 'food-producing animals' includes broilers, turkeys, pigs and calves for 2014–2015.

Consumption of polymyxins and resistance in indicator
E. coli from food-producing animals, 2014-2015
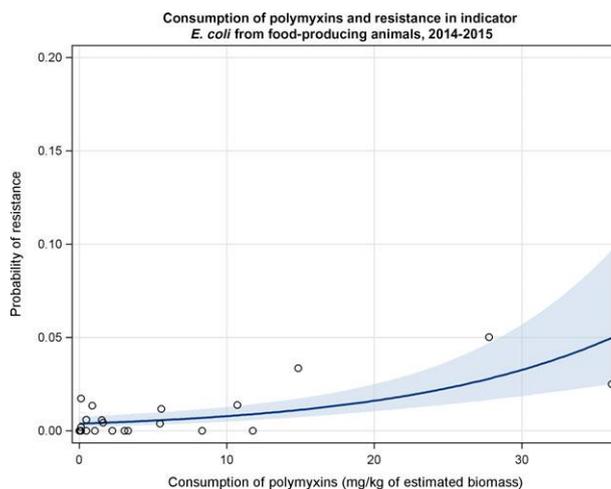
Dots represent the countries involved in the analysis. The category 'food-producing animals' include broilers, turkeys, pigs and calves for 2014–2015.

The scale used in the graph is adapted according to the range of probabilities of resistance observed, in order to best show the distribution of data points.

Figure 32: Logistic regression analysis curves of the consumption of polymyxins in food-producing animals and the probability of resistance to colistin in indicator *E. coli* from food-producing animals for 2014–2015 (see also Table 24)

8.3.2. In pigs and poultry

The estimated consumptions of polymyxins in pigs and in poultry were compared with the occurrence of resistance to colistin in indicator *E. coli* from slaughter pigs in 2015 for 18 countries and from poultry (broilers and turkeys) in 2014 for 27 countries, respectively.

Where detected, colistin resistance in indicator *E. coli* from pigs was typically reported at very low levels, whereas, in poultry, the levels of resistance observed were generally slightly higher to those in pigs, though still remaining low (Figure 33).

The association assessed between consumption of polymyxins and resistance to colistin in indicator *E. coli* in pigs in 2015 was significantly positive (Table 25). The association detected between consumption of polymyxins and resistance to colistin in indicator *E. coli* in poultry in 2014 was significantly positive (Table 25, Figure 33).

Table 25: Results of logistic regression for consumption of polymyxins in pigs and poultry, expressed in DDDvet/kg of estimated biomass/year and probability of resistance to polymyxins in indicator *E. coli* isolates from poultry (broilers and turkeys) and slaughter pigs (see also Figure 33)

Animal	Year	Countries included in the analysis	OR	95% PL CI	p-value
Indicator *E. coli*					
Poultry	2014	AT, BE, CH, CY, CZ, DE, DK, EE, ES, FI, FR, HR, HU, IE, IS, IT, LT, LU, LV, NL, NO, PL, PT, SE, SI, SK, UK (n = 27)	1.51	1.33–1.72	< 0.001
Pigs	2015	AT, BE, BG, CH, CY, DE, EE, FI, HR, HU, IE, LV, NO, PL, PT, RO, SE, SI, (n = 18)	1.72	1.13–3.06	0.011

OR: odds ratio; PL CI: profile likelihood confidence interval, 95%.
Note: OR varies from 0 to infinity. When OR equals 1 or CI includes 1, the association is not considered statistically-significant.
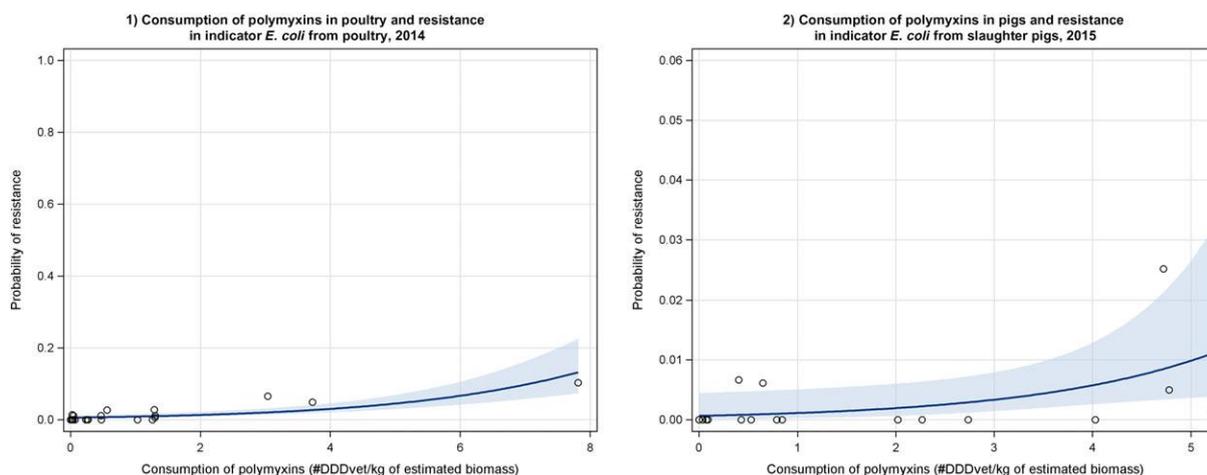
Dots represent the countries involved in the analysis. The poultry category includes broilers and turkeys.
The scale used in graph (2) was adapted according to the range of probabilities of resistance observed, in order to best show the distribution of data points.

Figure 33: Logistic regression analysis curves of the estimated consumption of polymyxins in pigs/ poultry and the probability of resistance to colistin in indicator *E. coli* isolates from (1) poultry in 2014 and (2) from slaughter pigs in 2015 (see Table 25)

8.4. Resistance to colistin in bacteria from animals *versus* resistance in bacteria from humans

Analysis of the available data on resistance to colistin in bacteria from animals and humans was rendered inconclusive due to incomparability of data from two different bacterial species, in this case indicator *E. coli* from animals vs invasive *K. pneumoniae* from humans. The issue of colistin resistance has been extensively addressed in relation to the recommendations to reduce use of colistin in food-producing animals (EMA/AMEG, 2016).

8.5. Key findings on polymyxins

- Polymyxins are categorised by AMEG in category 2, recommendations for reduction of its use in food-producing animals have been made. In the EU, the consumption of colistin is typically much higher in food-producing animals than in humans. The levels of use of colistin in food-producing animals varied markedly between reporting countries in three countries, colistin was not used in veterinary medicine. There was no correlation within country between consumption of polymyxins in humans and food-producing animals.
- In humans, data on colistin resistance was mainly available for invasive *K. pneumoniae* (characterised by the possibility or documentation of carbapenem resistance). A significant positive association was detected between consumption of polymyxins at the hospital (expressed in DDD per 1,000 inhabitants and per day) and resistance to polymyxins in invasive *K. pneumoniae* from humans in 2015.
- In food-producing animals, a significant positive association was also found between consumption of and resistance to colistin, whether considering the SIMR or the specific data on pigs and poultry.
- The structural lack of comparability between available data on colistin resistance in bacterial organisms from humans and food-producing animals did not allow fitting a multivariate model.

9. Macrolides

9.1. Consumption of macrolides by country

The population-weighted mean consumption of macrolides in humans and food-producing animals was 7.8 and 11.4 mg per kg of estimated biomass, respectively. The corresponding ranges were 1.5–19.8 (median 6.5) and 0–27.5 (median 4.9) mg per kg, respectively. Population-corrected consumption of macrolides in humans and food-producing animals by country is shown in Figure 34.

In 16 countries, the consumption was lower in food-producing animals than in humans; in three countries, the consumption was similar, and in the eight remaining countries, the consumption was higher in animals than in humans. There was no consumption of macrolides in food-producing animals in Iceland and Norway. Overall, the amount of macrolides consumed in food-producing animals and in humans varies between countries. There was no significant correlation between the levels of consumption of macrolides in humans and in food-producing animals (Spearman's rank correlation coefficient, $\rho = 0.32$) at the country level.
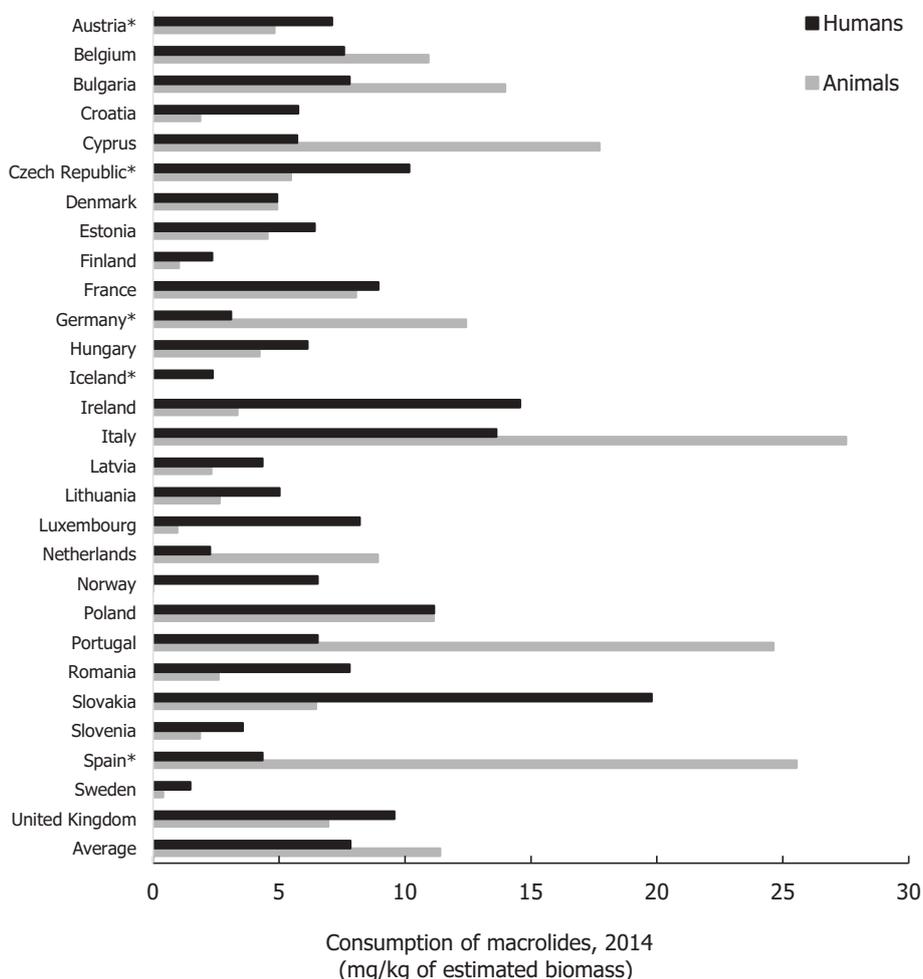
Asterisk (*) denotes that only community consumption was provided for human medicine. The population-weighted mean proportion (%) of the hospital sector from the 2014 total national consumption of antimicrobials for EU/EEA MSs that provided data for both sectors is 4.2%.

1) The estimates presented are crude and must be interpreted with caution. For limitations that hamper the comparison of consumption of antimicrobials in humans and animals, please see Section 14.

2) The average figure represents the population-weighted mean of data from included countries.

Figure 34: Population-corrected consumption of macrolides for humans and food-producing animals by country, EU/EEA MSs, 2014

9.2. Consumption of macrolides in humans and occurrence of resistance to macrolides in bacteria from humans

Macrolides are used for the treatment of infections caused by *Campylobacter* spp. (gastroenteritis) and Gram-positive bacteria, including respiratory infections suspected to be caused by *Mycoplasma pneumoniae*. Macrolides are considered by WHO (2017) as CIAs with the highest priority for human medicine.

9.2.1. *Campylobacter jejuni* and *C. coli*

Possible relationships between occurrence of resistance to macrolides in *C. jejuni* and *C. coli* isolates from humans and total (community and hospital) or community alone consumption of macrolides in humans in period 2013–2015 were analysed. No significant correlations were found between total or community alone (data not shown) consumption of macrolides in humans and resistance to macrolides in *C. jejuni* and *C. coli* from humans (Table 26).

Table 26: Results of logistic regression for total (community and hospital) consumption of macrolides in humans expressed in DDD per 1,000 inhabitants and per day, and the probability of resistance to macrolides in *C. jejuni* and *C. coli* from humans, EU/EEA, 2013–2015

Year	Countries included in the analysis	OR	95% PL CI	p-value
C. jejuni				
2013	AT, DK, EE, ES, FR, IT, LT, LU, MT, NL, NO, RO, SI, SK, UK (n = 15)	1.45	0.82–2.52	0.179
2014	AT, EE, ES, FR, IT, LT, LU, MT NL, NO, PT, RO, SI, SK (n = 14)	1.34	0.82–2.13	0.215
2015	AT, CY, DK, EE, ES, FI, FR, IS, IT, LT, LU, MT, NL, NO, PT, RO, SI, SK, UK (n = 19)	1.13	0.77–1.58	0.503
C. coli				
2013	AT, ES, FR, IT, LT, LU, MT, NL, SI, SK, UK (n = 11)	1.06	0.62–1.74	0.826
2014	AT, ES, FR, LT, LU, MT, NL, PT, SI, SK (n = 10)	0.84	0.32–1.80	0.688
2015	AT, CY, EE, ES, FI, FR, IT, LT, LU, MT, NL, PT, RO, SI, SK, UK (n = 16)	1.05	0.69–1.56	0.819

OR: odds ratio; PL CI: profile likelihood confidence interval, 95%.
OR varies from 0 to infinity. When OR equals 1 or CI includes 1, the association is not considered statistically-significant.

9.3. Comparison of consumption of macrolides with resistance to erythromycin in *C. jejuni* and *C. coli*

9.3.1. In food-producing animals

In order to explore potential relationships between consumption of macrolides and resistance to erythromycin in bacteria from animals, the SIMR to erythromycin in *C. jejuni* and *C. coli* from food-producing animals was compared with the consumption of macrolides in animals, at the country level. The SIMR to erythromycin in *C. coli* and *C. jejuni* combined resistance in those animal species in which those bacteria are prevalent and monitored, i.e. in broilers and pigs, and in broilers and cattle, respectively.

Resistance to erythromycin in *C. coli* was typically much greater than that recorded in *C. jejuni*. Although both associations were positive and of similar strength, the association assessed for *C. coli* proved to be statistically-significant, conversely to that for *C. jejuni* (Table 27, Figure 35).

Table 27: Results of logistic regression for consumption of macrolides in food-producing animals, expressed in mg/kg of estimated biomass/year, and probability of resistance to macrolides in *C. coli* from broilers and pigs and *C. jejuni* from broilers and cattle (see also Figure 35)

Year	Countries included in the analysis	OR	95% PL CI	p-value
C. jejuni				
2013	AT, CH, DE, DK, ES, FI, NL, SE (n = 8)[a]	1.09	0.96–1.25	0.165
C. coli				
2013	CH, ES, FR, HU, NL, UK (n = 6)[a]	1.15	1.10–1.21	< 0.001

OR: odds ratio; PL CI: profile likelihood confidence interval, 95%.
OR varies from 0 to infinity. When OR equals 1 or CI includes 1, the association is not considered statistically-significant.
(a): In the absence of 2013 resistance data, proxy data for years prior to 2013 may have been used.
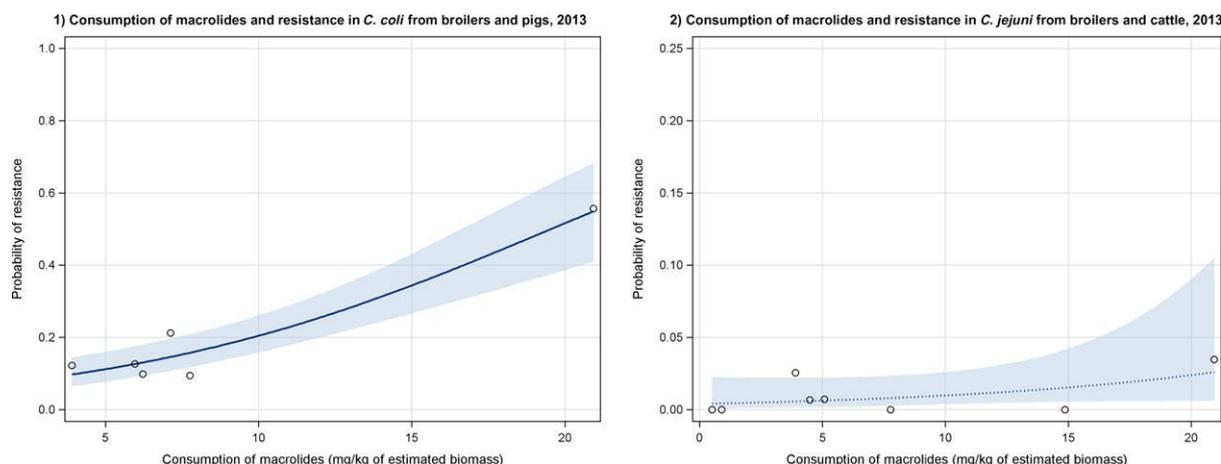
Dots represent the countries involved in the analysis.
Note: 1) The scale used in graph (2) was adapted according to the range of probabilities of resistance observed, in order to best show the distribution of data points.
2) In graph (2), the dashed curve means that the association is not significant.

Figure 35: Logistic regression analysis curves of the consumption of macrolides in food-producing animals and the probability of resistance to erythromycin in (1) *C. coli* from broilers and pigs and (2) *C. jejuni* from broilers and cattle in 2013 (see also Table 27)

9.3.2. In poultry

The estimated consumption of macrolides in poultry (expressed in DDDvet/kg of estimated biomass) was compared with the occurrence of resistance to erythromycin in *C. jejuni* from broilers in 2013 and from broilers and turkeys (SIMR) in 2014 (Table 28, Figure 36). For the year 2014, a higher number of countries was included in the analysis compared with 2013. Resistance in *C. jejuni* from turkeys was also accounted for in those countries where the turkey production sector is substantial. These two factors could explain why the association was significant for 2014 but not for 2013.

Table 28: Results of logistic regression for the estimated consumption of macrolides in poultry, expressed in DDDvet/kg of estimated biomass/year, and the probability of resistance to macrolides in *Campylobacter* spp. from poultry (see also Figure 36)

Animal	Year	Countries included in the analysis	OR[a]	95% PL CI	p-value
C. jejuni					
Poultry	2013[b]	AT, CZ, DE, DK, ES, FI, FR, HU, IS, NL, NO, SE, SI, UK (n = 14)	2.12	0.85–8.72	0.108
	2014	AT, BE, CY, CZ, DE, DK, ES, FI, FR, HR, HU, IE, IS, IT, LT, LV, NL, PL, PT, RO, SE, SI, SK, UK (n = 24)	1.24	1.01–1.46	0.013

OR: odds ratio; PL CI: profile likelihood confidence interval, 95%.
OR varies from 0 to infinity. When OR equals 1 or CI includes 1, the association is not considered statistically-significant.
The category 'poultry' includes broilers for 2013 and broilers and turkeys for 2014.
(a): OR estimated for 0.1-unit increment.
(b): In the absence of 2013 resistance data, proxy data for years prior to 2013 may have been used.

Consumption of macrolides in poultry and resistance in C. jejuni from broilers and turkeys, 2014
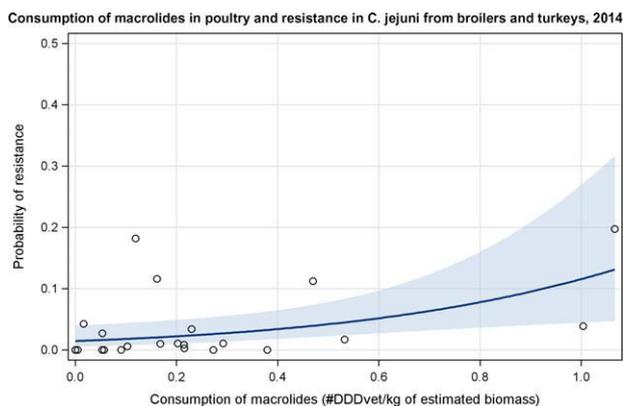
Dots represent the countries involved in the analysis.

Figure 36: Logistic regression analysis curves of the estimated consumption of macrolides in poultry and the probability of resistance to erythromycin in *C. jejuni* from broilers and turkeys in 2014 (see also Table 28)

9.4. Resistance to macrolides in bacteria from animals *versus* resistance to macrolides in bacteria from humans

Resistance in *Campylobacter* spp. isolates from humans to macrolides varies from low (0–8.7% for *C. jejuni,* 2015) to very high proportions (up to 55.7% for *C. coli*, 2014) (EFSA/ECDC, 2017). In food-producing animals, resistance in *Campylobacter* spp. isolates to macrolides varies between different EU/EEA countries and animal species.

9.4.1. Resistance in *C. jejuni* and *C. coli* from animals and humans

The analysis of the potential relationships between resistance to macrolides in *C. jejuni* from broilers (2013 and 2014), and turkeys (2014) and the corresponding ones in humans showed no significant associations (Table 29). Resistance to macrolides in *C. coli* from broilers was, however, significantly associated to resistance to macrolides in *C. coli* from humans in 2013 (Table 29, Figure 37). Data on *C. coli* from pigs were available in only four countries or less from both sectors for 2013–2015 and were therefore not analysed.

Table 29: Results of logistic regression for the probability of resistance to macrolides in *C. jejuni* and *C. coli* from broilers and from turkeys and humans (see also Figure 37)

Animal	Year	Countries	OR	95% PL CI	p-value
C. jejuni					
Broilers	2013	AT, CZ, DE, DK, ES, FI, FR, HU, IS, NL, NO, SE, SI, UK (n = 14)	1.55	0.88–2.58	0.095
	2014	AT, ES, FR, IT, LT, NL, PT, RO, SI, SK (n = 10)	0.99	0.83–1.12	0.926
Turkeys	2014	AT, ES, FR, IT, PT, RO (n = 6)	1.17	0.88–1.72	0.310
C. coli					
Broilers	2013	AT, ES, FR, NL, UK (n = 5)	1.05	1.02–1.08	0.001

OR: odds ratio; PL CI: profile likelihood confidence interval.
OR varies from 0 to infinity. When OR equals 1 or CI includes 1, the association is not considered statistically-significant.

Resistance to macrolides in *C. coli* from broilers and humans, 2013
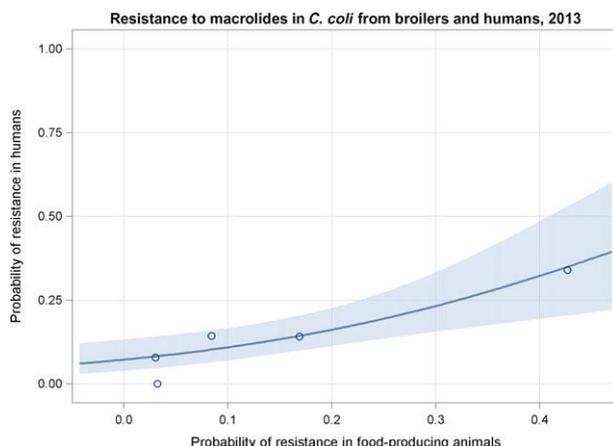
Dots represent countries included in the analysis.

Figure 37: Logistic regression analysis curves of the probability of resistance to macrolides in *C. coli* from food-producing animals (broilers) and humans, 2013 (see also Table 29)

9.5. Consumption of macrolides in food-producing animals *versus* resistance to macrolides in bacteria from humans

Possible relationships between the occurrence of resistance in *C. coli* and *C. jejuni* isolates from humans and the total consumption of macrolides in food-producing animals in 2013, 2014 and 2015 were assessed at the country level (Table 30), and generally, significant positive associations were discerned. For *C. jejuni*, significant positive associations were observed in each year under study. Considering *C. coli*, positive significant associations were only observed for the years 2013 and 2014.

Table 30: Results of logistic regression for consumption of macrolides in food-producing animals, expressed in mg/kg of estimated biomass/year, and probability of resistance to macrolides in *Campylobacter* spp. causing infections in humans (see also Figure 38)

Year	Countries included in the analysis	OR[a]	95% PL CI	p-value
C. coli				
2013[b]	AT, ES, FR, IT, LT, LU, NL, SI, SK, UK (n = 10)	1.12	1.06–1.18	< 0.001
2014	AT, ES, FR, LT, LU, NL, PT, SI, SK (n = 9)	1.13	1.09–1.16	< 0.001
2015	AT, CY, EE, FI, FR, IT, NL, PT, RO, SI, UK (n = 11)	1.05	0.98–1.13	0.174
C. jejuni				
2013[b]	AT, DK, EE, ES, FR, IT, LT, LU, NL, NO, SI, SK, UK (n = 13)	1.112	1.07–1.16	< 0.001
2014	AT, EE, ES, FR, IT, LT, LU, NL, NO, PT, RO, SI, SK (n = 13)	1.09	1.04–1.14	< 0.001
2015	AT, CY, DK, EE, FI, FR, IS, IT, NL, NO, PT, RO, SI, UK (n = 14)	1.07	1.01–1.12	0.016

OR: odds ratio; PL CI: profile likelihood confidence interval.
OR varies from 0 to infinity. When OR equals 1 or CI includes 1, the association is not considered statistically-significant.
(a): OR estimated for 0.1-unit increment.
(b): In the absence of 2013 resistance data, proxy data for years prior to 2013 may have been used.

9.6. Multivariate analysis

Data were insufficient to allow a multivariate analysis to be performed.

1) Consumption of macrolides in animals and resistance in *C. coli* from humans, 2013

2) Consumption of macrolides in animals and resistance in *C. coli* from humans, 2014

3) Consumption of macrolides in animals and resistance in *C. jejuni* from humans, 2013

4) Consumption of macrolides in animals and resistance in *C. jejuni* from humans, 2014

5) Consumption of macrolides in animals and resistance in *C. jejuni* from humans, 2015
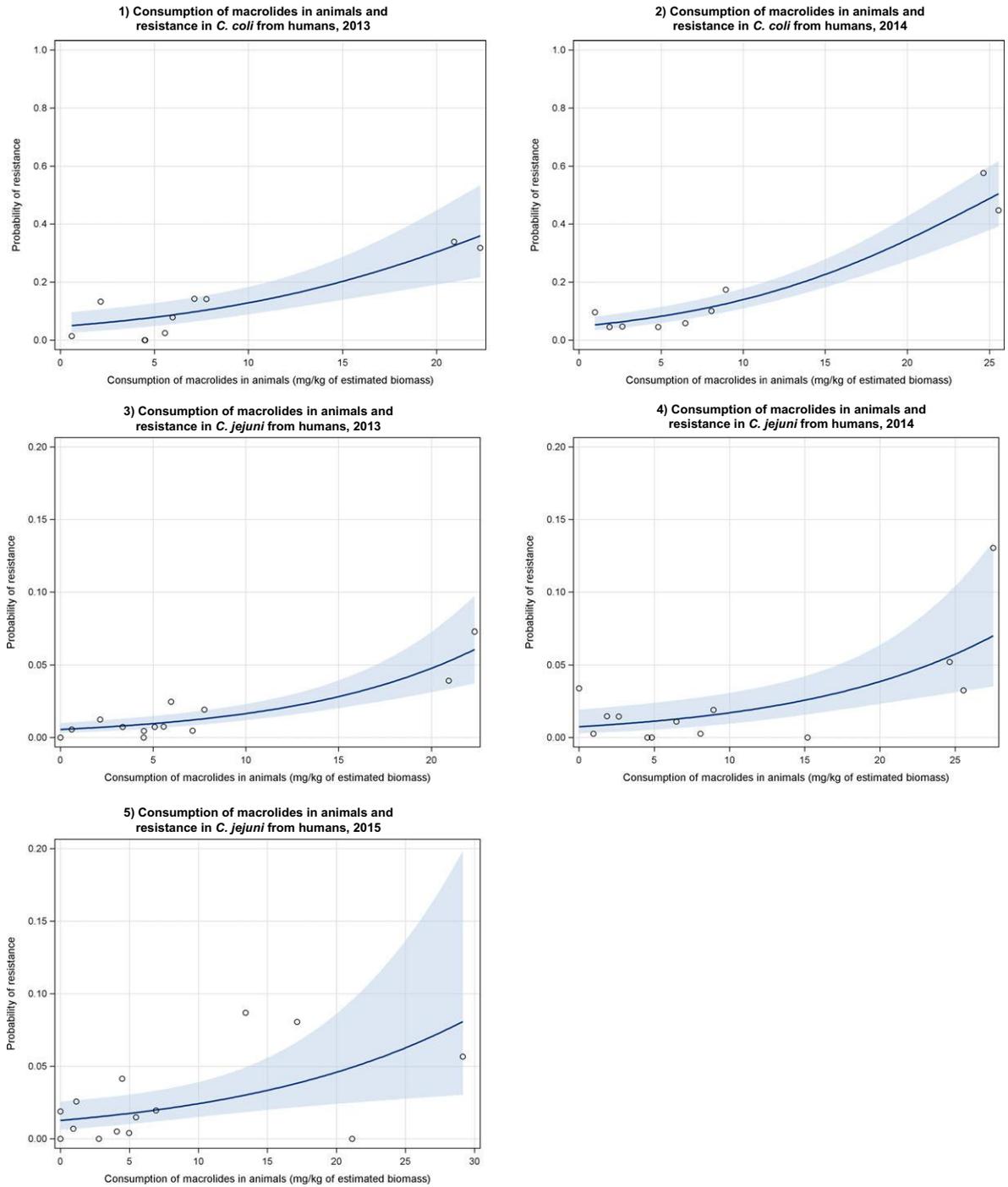
Dots represent the countries involved in the analysis.

The scale used in graphs (3), (4) and (5) is adapted according to the range of probabilities of resistance observed, in order to best show the distribution of data points.

Figure 38: Logistic regression analysis curves of the consumption of macrolides in food-producing animals and the probability of resistance to macrolides in *C. coli* and *C. jejuni* from humans, EU/EEA MSs, 2013–2015 (see also Table 30)

9.7.   Key findings on macrolides

- In human medicine, macrolides are nearly entirely used in the community. Overall, the consumption of macrolides (expressed in mg per kg of estimated biomass) was fairly similar in food-producing animals and in humans, although the quantities consumed varied between countries. No significant correlation was observed between macrolide consumption in humans and in food-producing animals at the MS level.
- No correlation was discerned between macrolide consumption in humans and macrolide resistance in *C. jejuni* and *C. coli* from humans.
- Statistically-significant positive associations were observed between the consumption of macrolides in food-producing animals and resistance in *C. coli* from both food-producing animals and humans. Similarly, resistance in *C. jejuni* from humans was strongly associated with the consumption of macrolides in food-producing animals.
- There was an insufficient number of countries providing data on *C. coli* from pigs for any analyses of resistance to this class of antimicrobial to be undertaken.
- Data were insufficient to allow a multivariate analysis to be performed.

10.   Tetracyclines

10.1.   Consumption of tetracyclines by country

The population-weighted mean consumption of tetracyclines in humans and food-producing animals was 3.6 and 50.6 mg per kg of estimated biomass, respectively. The corresponding ranges were 0.3–13.5 (median 1.8) and 0.1–151.5 (median 25.1) mg per kg, respectively. Population-corrected consumption of tetracyclines in humans and food-producing animals by country is shown in Figure 39.

In 24 countries, the amounts of tetracyclines consumed in food-producing animals outweighed that of humans, mostly by far, but in four countries the consumption in animals was lower. Also, the variation between countries in the quantities of tetracyclines consumed in food-producing animals was very wide. There was no significant correlation within country between consumption of tetracyclines in humans and food-producing animals (Spearman's rank correlation, $\rho = -0.36$).
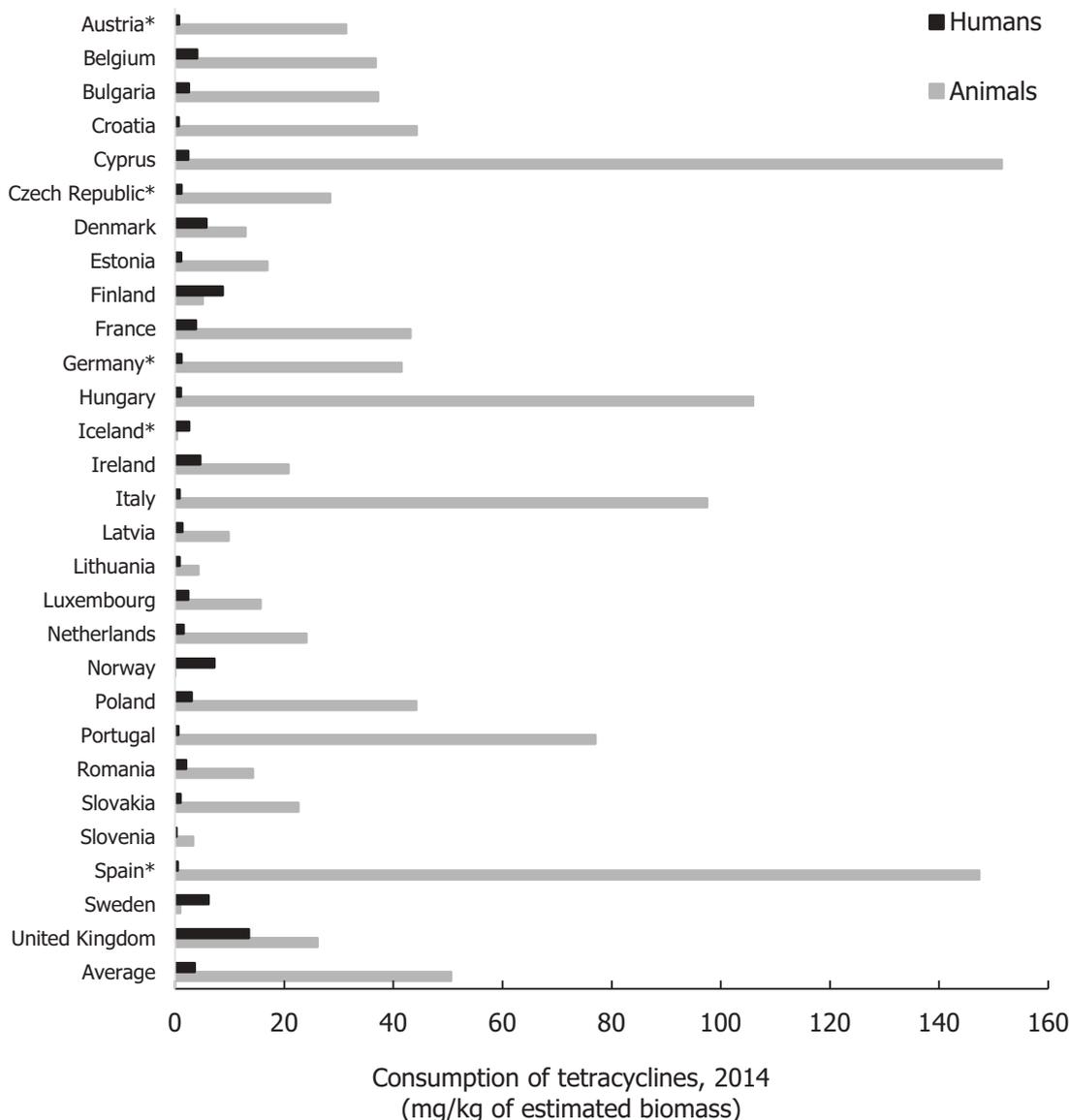
Asterisk (*) denotes that only community consumption was provided for human medicine. The population-weighted mean proportion (%) of the hospital sector from the 2014 total national consumption of antimicrobials for EU/EEA MSs that provided data for both sectors is 2.9%.
Notes: 1) The estimates presented are crude and must be interpreted with caution. For limitations that hamper the comparison of consumption of antimicrobials in humans and animals, please see Section 14.
2) The average figure represents the population-weighted mean of data from included countries.

Figure 39: Population-corrected consumption of tetracyclines for humans and food-producing animals by country, EU/EEA MSs, 2014

10.2. Consumption of tetracyclines in humans and occurrence of resistance to tetracyclines in bacteria from humans

Tetracyclines are broad-spectrum antimicrobial agents used for treatment of infections caused by both Gram-negative and Gram-positive bacteria. Tetracyclines are generally not used for treatment of *E. coli* infections in humans, and resistance to tetracyclines in invasive *E. coli* isolates from humans is not under surveillance.

10.2.1. *Salmonella* Enteritidis, *S.* Typhimurium, monophasic *S.* Typhimurium and *S.* Infantis isolates

Total (community and hospital) consumption and community-only consumption of tetracyclines were significantly associated with tetracycline resistance in *S.* Enteritidis in humans for the years 2013 and 2015 (Table 31, Figure 40).

Table 31: Results of logistic regression for total (community and hospital) consumption and for community consumption of tetracyclines in humans, expressed in DDD per 1,000 inhabitants and per day, and probability of resistance to tetracyclines in salmonella serovars from humans, EU/EEA, 2013–2015 (see also Figure 40)

| Year	Countries included in the analysis	OR	95% PL CI	p-value
S. Enteritidis – total consumption (community and hospitals)				
2013	AT, BE, EE, EL, ES, FI, FR, HU, IE, IT, LT, LU, NL, NO, RO, SI, SK, UK (n = 18)	1.31	1.01–1.67	0.038
2014	AT, BE, DE, ES, FI, FR, HU, IE, LT, LU, NL, NO, PT, RO, SI, SK, UK (n = 17)	1.04	0.65–1.44	0.983
2015	AT, DE, EE, EL, ES, FI, FR, IE, IT, LT, LU, NL, NO, PT, RO, SI, SK, UK (n = 18)	1.43	1.09–1.86	0.001
S. Enteritidis – community consumption				
2013	AT, BE, EE, EL, ES, FI, FR, HU, IE, IT, LT, LU, NL, NO, RO, SI, SK, UK (n = 18)	1.29	1.08–1.55	0.005
2014	AT, BE, DE, ES, FI, FR, HU, IE, LT, LU, NL, NO, PT, RO, SI, SK, UK (n = 17)	1.03	0.86–1.22	0.771
2015	AT, DE, EE, EL, ES, FI, FR, IE, IT, LT, LU, NL, NO, PT, RO, SI, SK, UK (n = 18)	1.27	1.09–1.49	0.002
S. Typhimurium – total consumption (community and hospitals)				
2013	AT, BE, DK, EE, EL, ES, FI, FR, HU, IE, IT, LT, LU, NL, NO, RO, SI, SK, UK (n = 19)	0.95	0.75–1.22	0.696
2014	AT, BE, DE, DK, ES, FI, FR, HU, IE, LT, LU, NL, NO, PT, RO, SI, SK, UK (n = 18)	0.95	0.71–1.26	0.726
2015	AT, DE, DK, EE, EL, ES, FI, FR, HU, IE, IT, LT, LU, NL, NO, PT, RO, SI, SK, UK (n = 20)	0.98	0.72–1.32	0.884
S. Typhimurium – community consumption				
2013	AT, BE, DK, EE, EL, ES, FI, FR, HU, IE, IT, LT, LU, NL, NO, RO, SI, SK, UK (n = 19)	0.84	0.78–0.91	< 0.001
2014	AT, BE, DE, DK, ES, FI, FR, HU, IE, LT, LU, NL, NO, PT, RO, SI, SK, UK (n = 18)	0.93	0.86–1.00	0.056
2015	AT, DE, DK, EE, EL, ES, FI, FR, HU, IE, IT, LT, LU, NL, NO, PT, RO, SI, SK, UK (n = 20)	1.01	0.93–1.10	0.778
Monophasic S. Typhimurium – total consumption (community and hospitals)				
2013	AT, DK, EL, ES, FR, HU, IE, IT, LU, NL (n = 10)	1.11	0.84–1.48	0.481
2014	AT, DK, ES, FR, HU, IE, IT, LU, NL, PT (n = 10)	1.26	0.67–2.56	0.494
2015	AT, DK, EE, ES, FR, HU, IE, IT, LU, NL, PT (n = 11)	1.20	0.96–1.54	0.121
S. Infantis – total consumption (community and hospitals)				
2013	AT, BE, ES, FI, FR, IE, IT, LT, NL, RO, SI, SK, UK (n = 13)	0.70	0.35–1.24	0.256
2014	AT, BE, DE, DK, ES, FR, HU, IT, LT, NL, SI, SK, UK (n = 13)	0.83	0.44–1.42	0.506
2015	AT, DE, EE, ES, FI, FR, HU, NL, SI, SK, UK (n = 11)	0.73	0.43–1.14	0.191

OR: odds ratio; PL CI: profile likelihood confidence interval, 95%.
OR varies from 0 to infinity. When OR equals 1 or CI includes 1, the association is not considered statistically-significant.

1) Consumption of tetracyclines and resistance in *S.* Enteritidis from humans, 2013

2) Consumption of tetracyclines and resistance in *S.* Enteritidis from humans, 2015
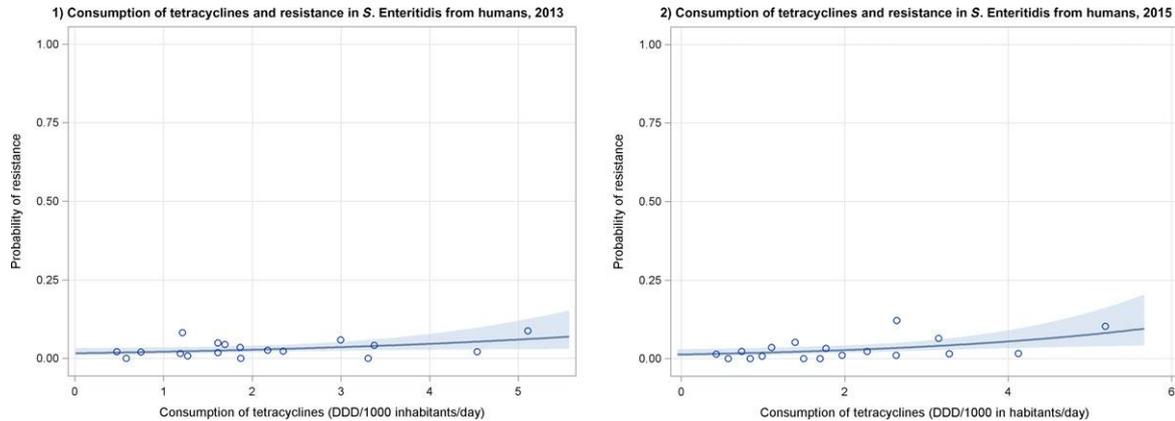
Dots represent countries included in the analysis.

Figure 40: Logistic regression analysis curves of the total (community and hospital) consumption of tetracyclines in humans expressed in DDD per 1,000 inhabitants and per day, and the probability of resistance to tetracyclines in *S.* Enteritidis isolates from humans, EU/EEA, 2013 and 2015 (see also Table 31)

When the total (community and hospital) consumption of tetracyclines was correlated with resistance to tetracyclines of *S.* Typhimurium*,* monophasic *S.* Typhimurium and *S.* Infantis, no significant association was found for the years 2013, 2014 and 2015 (Table 31). Only in 2013, was consumption of tetracyclines in the community correlated to the occurrence of resistance in *S.* Typhimurium isolates (Table 31).

10.2.2. *Campylobacter jejuni* and *C. coli*

Data on the occurrence of resistance to tetracyclines in *C. coli* and *C. jejuni* from cases of human infections were reported for 2015 by 14 and 15 countries, respectively. In 2015, the number of isolates obtained per country varied from 39 to 844 for *C. coli* and from 31 to 4,472 for *C. jejuni*. For the same year, occurrence of resistance to tetracyclines varied in *C. coli* from 29.4% to 95.3% and in *C. jejuni* from 13.2% to 81.8%.

In order to assess potential association between the consumption of tetracyclines and the occurrence of resistance to tetracyclines in *Campylobacter* spp., the consumption of tetracyclines in total (community and hospital) and in the community alone, was analysed against the occurrence of tetracycline resistance in *C. coli* and *C. jejuni* for the years 2013–2015. When total consumption data were analysed against occurrence of resistance of *C. coli* and *C. jejuni,* no significant association was found for the years 2013–2015 (Table 32).

Table 32: Results of logistic regression for total (community and hospital) consumption of tetracyclines in humans expressed in DDD per 1,000 inhabitants and per day, and the probability of resistance to tetracyclines in *Campylobacter jejuni* and *C. coli* isolates from humans, EU/EEA, 2013–2015

Year	Countries included in the analysis	OR	95% PL CI	p-value
C. jejuni - total consumption (community and hospitals)				
2013	AT, DK, EE, ES, IT, LU, NL, NO, RO, SI, SK, UK (n = 12)	0.76	0.47–1.14	0.211
2014	AT, EE, ES, FR, IT, LT, LU, NL, NO, PT, RO, SI, SK (n = 13)	0.70	0.43–1.13	0.150
2015	AT, CY, DK, EE, ES, FI, FR, IT, LT, LU, NL, NO, PT, RO, SI (n = 15)	0.79	0.58–1.05	0.118
C. jejuni - community consumption				
2013	AT, DK, EE, ES, IT, LU, NL, NO, RO, SI, SK, UK (n = 12)	0.87	0.81–0.94	< 0.001
2014	AT, EE, ES, FR, IT, LT, LU, NL, NO, PT, RO, SI, SK (n = 13)	1.03	0.98–1.08	0.185
2015	AT, CY, DK, EE, ES, FI, FR, IT, LT, LU, NL, NO, PT, RO, SI (n = 15)	0.94	0.91–0.98	0.006
C. coli - total consumption (community and hospitals)				
2013	AT, ES, IT, LU, NL, SI, SK (n = 7)	0.80	0.25–2.55	0.704
2014	AT, ES, FR, LU, NL, PT, SI, SK (n = 8)	0.69	0.32–1.44	0.329
2015	AT, CY, EE, ES, FI, FR, IT, LT, LU, NL, PT, RO, SI, SK (n = 14)	1.09	0.72–1.73	0.672
C. coli - community consumption				
2013	AT, ES, IT, LU, NL, SI, SK (n = 7)	0.98	0.76–1.28	0.907
2014	AT, ES, FR, LU, NL, PT, SI, SK (n = 8)	0.60	0.52–0.71	< 0.001
2015	AT, CY, EE, ES, FI, FR, IT, LT, LU, NL, PT, RO, SI, SK (n = 14)	0.97	0.85–1.11	0.633

OR: odds ratio; PL CI: profile likelihood confidence interval, 95%.
OR varies from 0 to infinity. When OR equals 1 or CI includes 1, the association is not considered statistically-significant.

Statistically-significant associations were found between community consumption of tetracyclines and resistance to tetracyclines. These were found in 2013 and in 2015 for *C. jejuni* and in 2014 for *C. coli* (Table 32).

10.3. Comparison of consumption of tetracyclines in animals with resistance to tetracycline in bacteria from animals

10.3.1. In food-producing animals

In order to investigate possible relationships between the consumption of tetracyclines and tetracycline resistance, the SIMR to tetracyclines in indicator *E. coli*, *Salmonella* spp., *C. jejuni* and *C. coli* was compared with the consumption of tetracyclines in animals (expressed in mg per kg of estimated biomass) for 2013 and 2014–2015 (average consumption over 2014 and 2015), at the country level (Table 33, Figure 41). The category 'food-producing animals' includes broilers, pigs and cattle for 2013 and broilers, turkeys, pigs and calves for 2014–2015.

Marked variations in tetracycline resistance in indicator *E. coli*, *Salmonella* spp., *C. jejuni* and *C. coli* were typically observed between the countries included in the analysis. The consumption of tetracyclines ranged between a few units and up to 150 mg per kg of estimated biomass. Statistically-significant positive associations between tetracycline resistance in indicator *E. coli,* and *Salmonella* spp., and tetracycline consumption in animals were typically observed in 2013 and 2014–2015. A statistically-significant positive association between tetracycline resistance and tetracycline consumption was observed in 2013 for *C. jejuni,* but not for *C. coli*.

Table 33:   Results of logistic regression for consumption of tetracyclines in food-producing animals, expressed in mg/kg of estimated biomass/year, and probability of resistance to tetracyclines in bacteria from food-producing animals (see also Figure 41)

Year	Countries included in the analysis	OR	95% PL CI	p-value
Indicator *E. coli*				
2013[a]	AT, BE, CH, DE, DK, ES, FI, HU, NL, PL, SE (n = 11)	1.02	1.01–1.03	0.001
2014–2015	AT, BE, BG, CY, CZ, DE, DK, EE, ES, FI, FR, HR, HU, IE, IT, LT, LV, NL, NO, PL, PT, RO, SE, SI, SK, UK (n = 26)	1.02	1.01–1.03	< 0.001
***Salmonella* spp.**				
2013[a]	BE, DE, DK, ES, IE, IT, NL, PL, UK (n = 9)	1.03	1.02–1.05	0.047
2014–2015	BE, CZ, DK, ES, FR, HR, IT, PT (n = 8)	1.01	1.00–1.02	0.049
C. jejuni				
2013[a]	AT, CH, DE, DK, ES, FI, NL, SE (n = 8)	1.03	1.01–1.06	0.007
C. coli				
2013[a]	CH, ES, FR, HU, NL, UK (n = 6)	1.02	0.998–1.06	0.076

OR: odds ratio; PL CI: profile likelihood confidence interval, 95%.

OR varies from 0 to infinity. When OR equals 1 or CI includes 1, the association is not considered statistically-significant.

Regarding resistance data, the category 'food-producing animals' includes broilers, pigs and cattle for 2013, and broilers, turkeys, pigs and calves for 2014–2015.

(a): In the absence of 2013 resistance data, proxy data for years prior to 2013 may have been used.
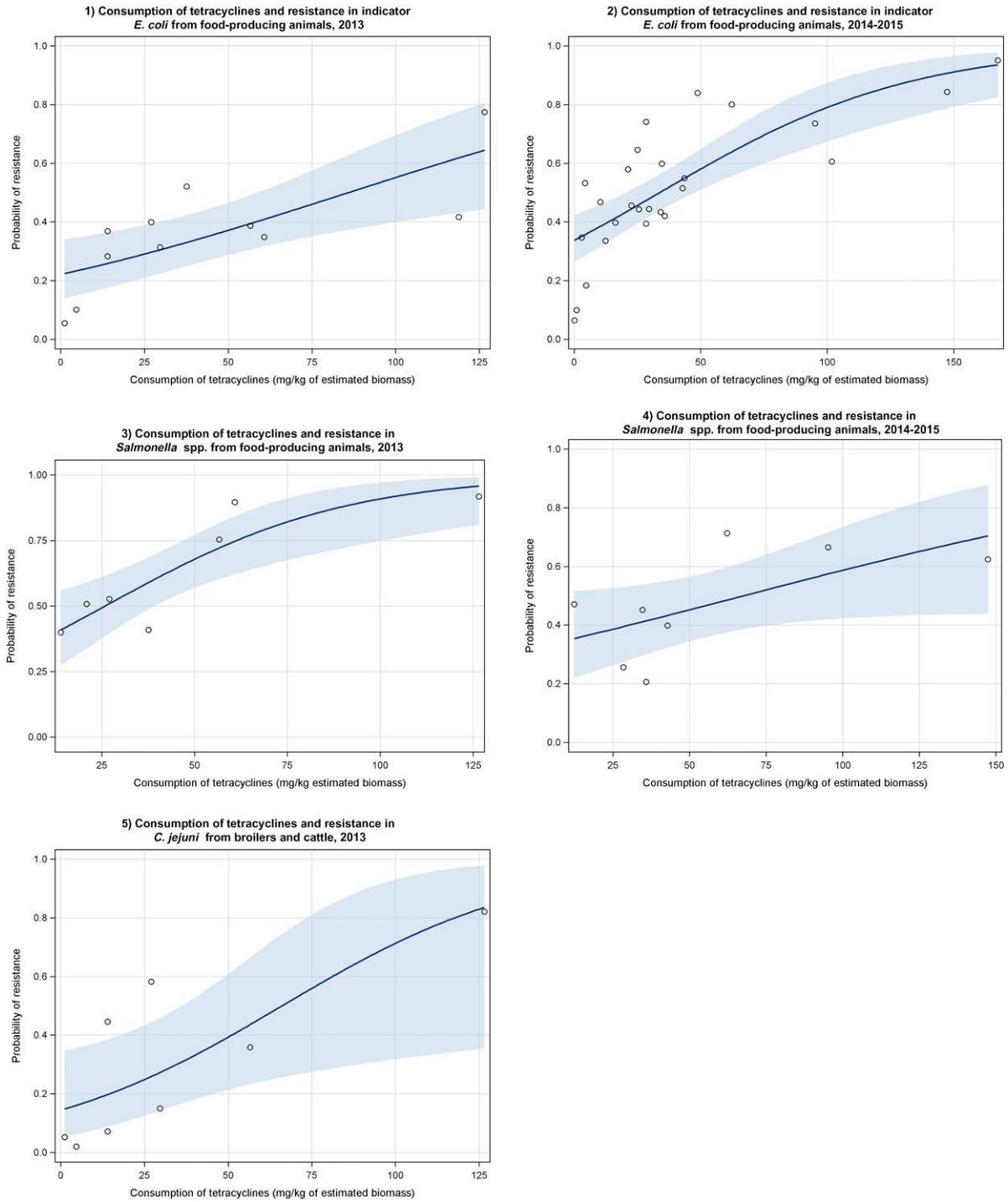
Dots represent the countries included in the analysis.
Category 'food-producing animals' includes broilers, cattle and pigs for 2013 and broilers, turkeys, pigs and calves for 2014–2015.

Figure 41: Logistic regression analysis curves of the consumption of tetracyclines in food-producing animals and the probability of resistance to tetracyclines in indicator *E. coli* from food-producing animals in (1) 2013 and (2) 2014–2015, in *Salmonella* spp. from food-producing animals in (3) 2013 and (4) 2014–2015, and in (5) *C. jejuni* from broilers and cattle in 2013 (see also Table 33)

10.3.2. In pigs and poultry

The estimated consumption of tetracyclines in pigs (expressed in DDDvet/kg of estimated biomass) was compared with the occurrence of resistance to tetracyclines in indicator *E. coli* from slaughter pigs in 2013 and 2015 for 12 and 18 reporting countries, respectively. Both associations were significantly positive (Table 34, Figure 42).

Table 34: Results of logistic regression for the estimated consumption of tetracyclines in pigs and poultry, expressed in DDDvet/kg of estimated biomass/year, and the probability of resistance to tetracyclines in bacteria from slaughter pigs and poultry (see also Figure 42)

Animal	Year	Countries included in the analysis	OR	95% PL CI	p-value
Indicator *E. coli*					
Slaughter pigs	2013[a]	AT, BE, DE, DK, ES, FI, FR, HU, NL, PL, SE, UK (n = 12)	1.21	1.09–1.35	< 0.001
	2015	AT, BE, BG, CH, CY, DE, EE, FI, HR, HU, IE, LV, NO, PL, PT, RO, SE, SI (n = 18)	1.36	1.22–1.53	< 0.001
Poultry	2014	AT, BE, BG, CY, CZ, DE, DK, EE, ES, FI, FR, HR, HU, IE, IT, LT, LV, NL, NO, PL, PT, RO, SE, SI, SK, UK (n = 26)	1.57	1.27–1.96	< 0.001
***Salmonella* spp.**					
Poultry	2013[a]	AT, BE, CZ, DE, DK, ES, FR, HU, IE, IT, NL, PL, SI, SK, UK (n = 15)[b]	1.08	0.78–1.47	0.643
	2014	AT, BE, BG, CY, CZ, DE, DK, ES, FR, HR, HU, IE, IS, IT, NL, PL, PT, RO, SI, SK, UK (n = 21)	1.35	0.99–1.87	0.057
C. jejuni					
Poultry	2014	AT, BE, CY, CZ, DE, DK, ES, FI, FR, HR, HU, IE, IS, IT, LT, LV, NL, PL, PT, RO, SE, SI, SK, UK (n = 24)	1.60	1.26–2.04	< 0.001

OR: odds ratio; PL CI: profile likelihood confidence interval, 95%.
OR varies from 0 to infinity. When OR equals 1 or CI includes 1, the association is not considered statistically-significant.
(a): In the absence of 2013 resistance data, proxy data for years prior to 2013 may have been used.
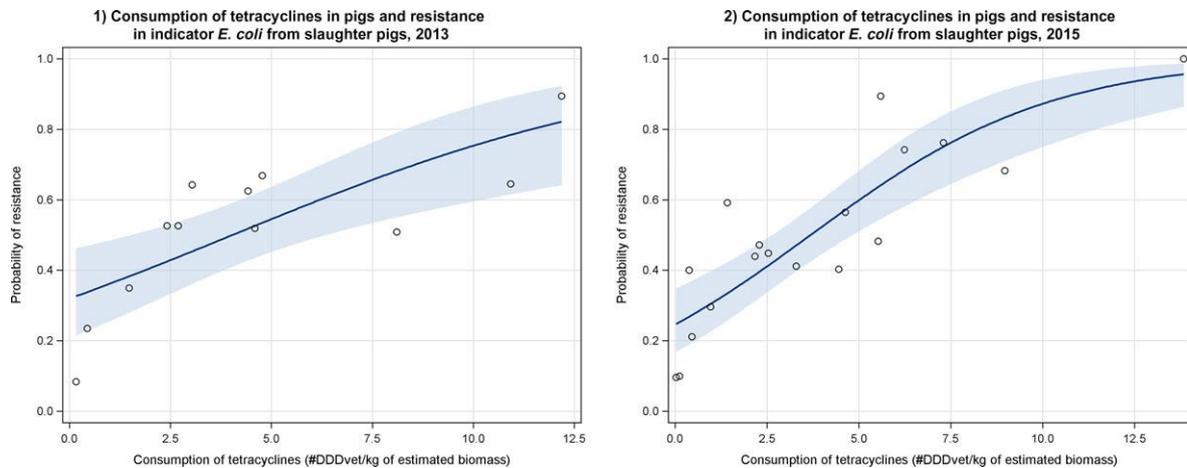
Dots represent the countries included in the analysis.

Figure 42: Logistic regression analysis curves of the estimated consumption of tetracyclines in pigs and the probability of resistance to tetracyclines in indicator *E. coli* from slaughter pigs in (1) 2013 and (2) 2015 (see also Table 34)

The estimated consumption of tetracyclines in poultry, expressed in DDDvet/kg of estimated biomass, was compared with the occurrence of resistance to tetracyclines in indicator *E. coli*, *Salmonella* spp. and *C. jejuni* from broilers and turkeys in 2013 and 2014 (Table 34). Both data on ciprofloxacin resistance to and consumption of tetracyclines in poultry were available together from 15 and 20 countries for *Salmonella* spp. in 2013 and 2014, respectively and in 26 countries for indicator *E. coli* in 2014 and in 24 countries for *C. jejuni* in 2014 (Figure 43).

Consumption of tetracyclines was positively associated with resistance to tetracyclines in indicator *E. coli*. and *C. jejuni* in 2014, whereas for *Salmonella* spp. isolates in 2013 and 2014, there was no significant association.
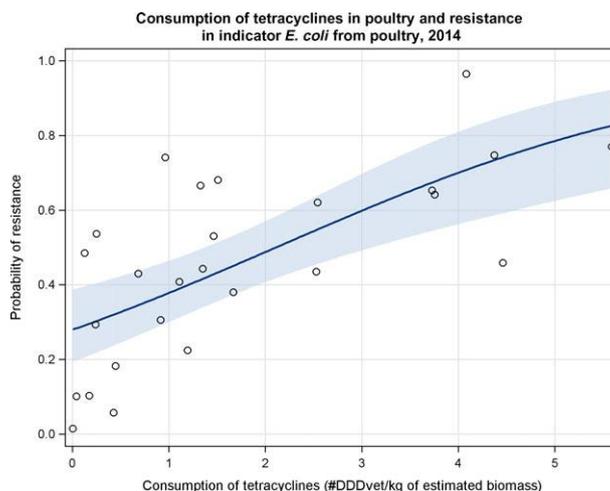
Dots represent the countries included in the analysis.

Figure 43: Logistic regression analysis curves of the estimated consumption of tetracyclines in poultry and the probability of resistance to tetracyclines in indicator *E. coli* from poultry in 2014 (see also Table 34)

10.4. Resistance to tetracyclines in bacteria from animals *versus* resistance to tetracyclines in bacteria from humans

Occurrence of resistance to tetracyclines of *Salmonella* spp. and *Campylobacter* spp. has been detected both in humans and food-producing animals but varies markedly among EU/EEA MS.

10.4.1. Resistance in *S.* Typhimurium, *S.* Enteritidis and *S.* Infantis from animals and humans

In 2013–2015, few countries provided data from both food-producing animals and humans for the different *Salmonella* spp. serovars and this prevented many of the correlations from being made (data not shown). When at least five countries provided data then correlation was performed. In 2013, resistance of *S.* Typhimurium to tetracyclines from pigs was not correlated to resistance to tetracyclines of *S.* Typhimurium from humans (Table 35).

Table 35: Results of logistic regression for the probability of resistance/resistance to tetracyclines in *Salmonella* spp. and salmonella serovars from food-producing animals, pigs and broilers and humans (see also Figures 44, 45 and 46)

Animal	Year	Countries	OR	95% PL CI	p-value
Salmonella spp.					
FPA	2014–2015	BE, DK, ES, FR, IT, PT (n = 6)	3.70	2.21–6.20	< 0.001
S. Typhimurium					
Pigs	2013	BE, DK, IE, NL, UK (n = 5)	1.01	0.93–1.09	0.894
S. Enteritidis					
Broilers	2013	AT, BE, ES, FR, HU, IT, NL, RO, SK (n = 9)	0.98	0.88–1.08	0.627
	2014	AT, BE, HU, NL, RO (n = 5)	1.05	1.03–1.06	< 0.001
S. Infantis					
Broilers	2013	AT, BE, ES, IT, NL, RO, SI, SK (n = 8)	1.03	1.01–1.06	0.029
	2014	AT, BE, DE, HU, IT, NL, SI, SK (n = 8)	1.03	1.01–1.07	0.017

FPA: food-producing animals; OR: odds ratio; PL CI: profile likelihood confidence interval.
OR varies from 0 to infinity. When OR equals 1 or CI includes 1, the association is not considered statistically-significant.

Resistance of *S.* Enteritidis to tetracyclines from broilers in 2014 but not in 2013 was significantly correlated to resistance to tetracyclines of the same bacterium from humans (Table 35, Figure 44). When omitting the largest value in 2014, the correlation becomes non-significant.
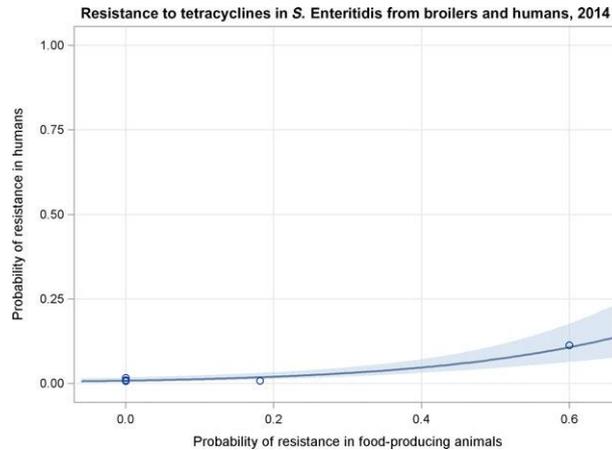
Dots represent countries included in the analysis.

Figure 44: Logistic regression analysis curves of the probability of resistance to tetracyclines in *S.* Enteritidis from broilers and humans, 2014 (see also Table 35)

Resistance of *S.* Infantis to tetracyclines from broilers (2013 and 2014) was significantly correlated to resistance to tetracyclines of the same bacterium from humans (Table 35, Figure 45).
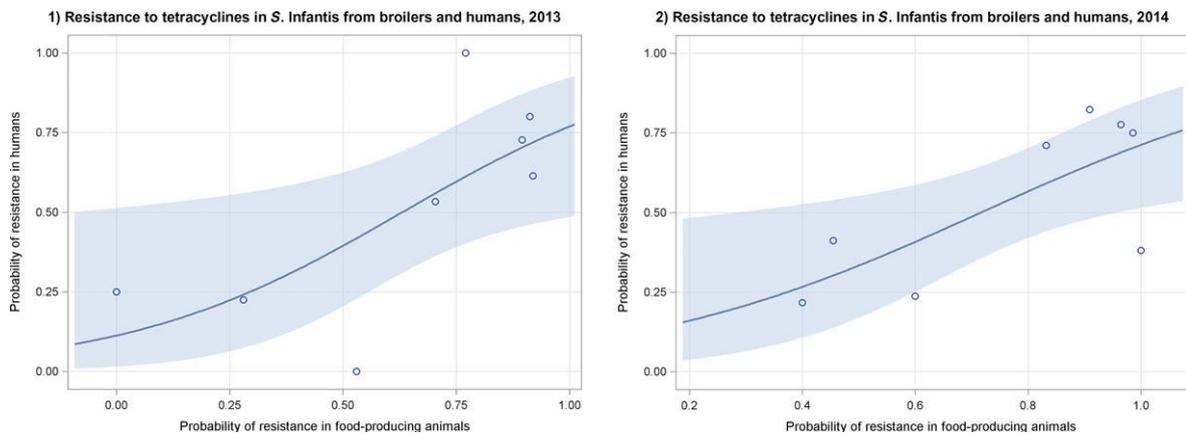
Dots represent countries included in the analysis.

Figure 45: Logistic regression analysis curves of the probability of resistance to tetracyclines in *S.* Infantis from broilers and humans, 2013–2014 (see also Table 35)

Using aggregate data for 2014 and 2015, resistance of *Salmonella* spp. to tetracyclines from humans significantly correlated to resistance to tetracyclines of *Salmonella* spp. from food-producing animals (SIMR) (Figure 46).

Resistance to tetracyclines in *Salmonella* spp.
from animals and humans, 2014–2015
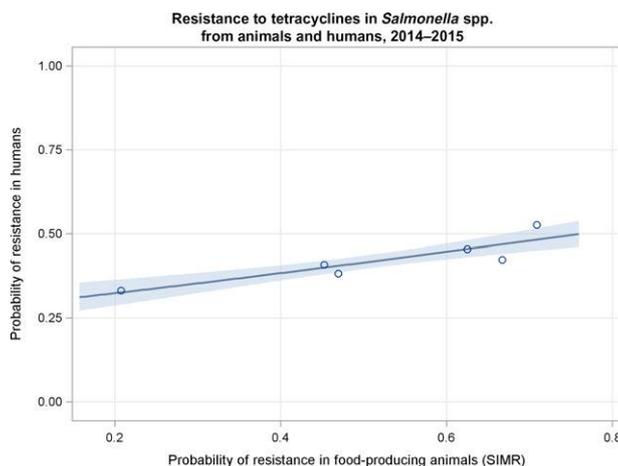
Dots represent countries included in the analysis.

Figure 46: Logistic regression analysis curves of the probability of resistance to tetracyclines in *Salmonella* spp. from humans and the probability of resistance to tetracyclines in *Salmonella* spp. (SIMR) from food-producing animals (combined data for 2014–2015) (see also Table 35)

10.4.2. Resistance in *Campylobacter jejuni* from animals and humans

For *C. jejuni*, resistance to tetracyclines from broilers in 2013 and 2014 and turkeys in 2014 was significantly correlated to resistance to tetracyclines from humans (Table 36, Figure 47). [Data on *Campylobacter coli* was only available from four countries or less from both sectors for 2013–2015 and were therefore not included].

Table 36: Results of logistic regression for the probability of resistance to tetracyclines in *Campylobacter jejuni* from food-producing animals and humans (see also Figure 47)

Animal	Year	Countries	OR	95% PL CI	p-value
C. jejuni					
Broilers	2013	AT, DK, ES, NL, NO, SI, UK (n = 7)	1.04	1.03–1.05	< 0.001
	2014	AT, ES, FR, IT, LT, NL, PT, RO, SI, SK (n = 10)	1.04	1.03–1.05	< 0.001
Turkeys	2014	AT, ES, FR, IT, PT, RO (n = 6)	1.04	1.03–1.05	< 0.001

OR: odds ratio; PL CI: profile likelihood confidence interval.
OR varies from 0 to infinity. When OR equals 1 or CI includes 1, the association is not considered statistically-significant.

1) Resistance to tetracyclines in *C. jejuni* from broilers and humans, 2013

2) Resistance to tetracyclines in *C. jejuni* from broilers and humans, 2014

3) Resistance to tetracyclines in *C. jejuni* from turkeys and humans, 2014
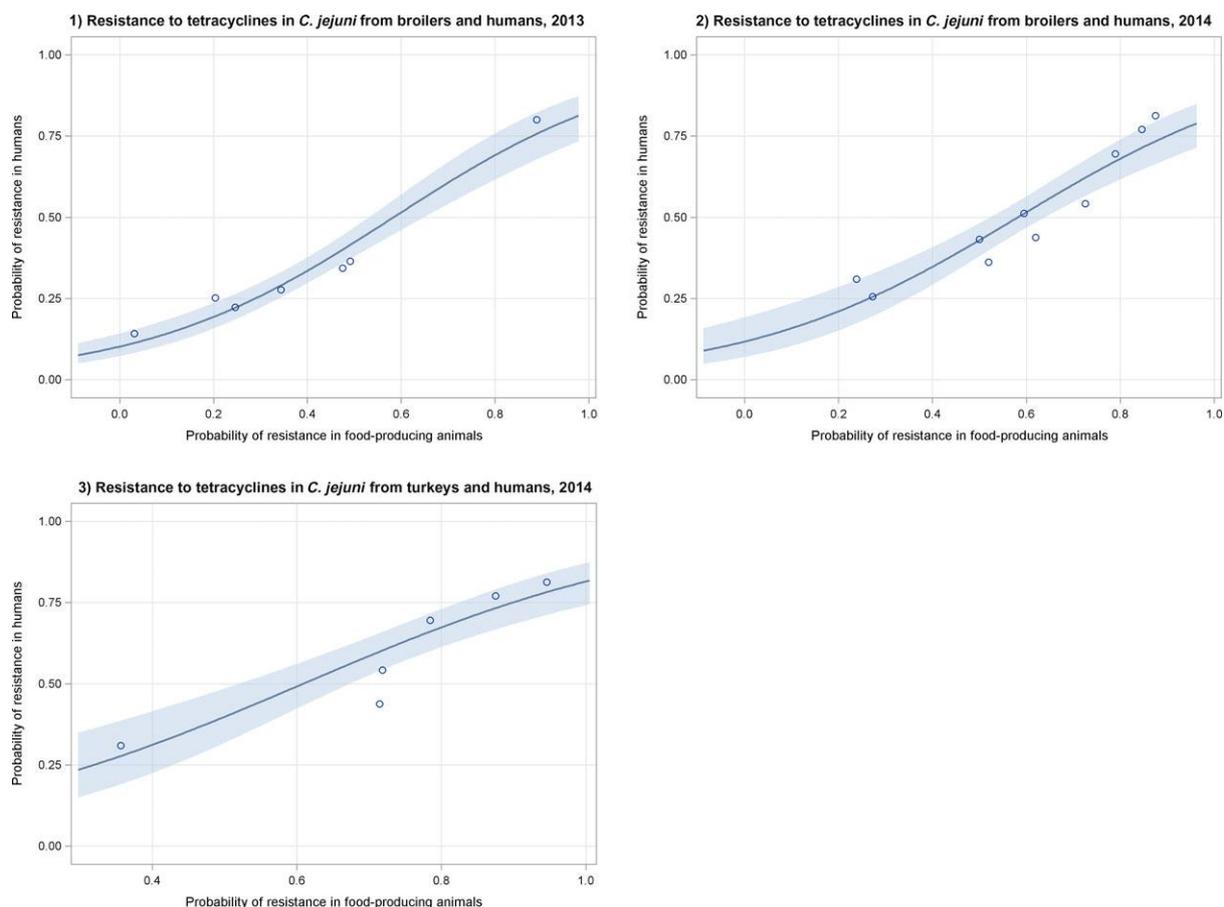
Dots represent countries included in the analysis.

Figure 47: Logistic regression analysis curves of the probability of resistance to tetracyclines in *Campylobacter jejuni* from broilers, turkeys and humans, 2013–2014 (see also Table 36)

10.5. Consumption of tetracyclines in food-producing animals *versus* resistance to tetracyclines in bacteria from humans

Data on resistance to tetracyclines were obtained for *Salmonella* spp., *S*. Typhimurium, *S*. Enteritidis, *S*. Infantis, monophasic *S*. Typhimurium, *C. coli* and *C. jejuni* from human infections and compared with the consumption of tetracyclines in animals in 2013, 2014 and 2015 (Table 37).

For *Campylobacter* spp., data available were more limited than for *Salmonella* spp. Positive significant associations between AMC of and resistance to tetracyclines were observed in *C. coli* and *C. jejuni* isolates for both years 2013 and 2014. Although positive associations were also discerned in *C. coli* and *C. jejuni* isolates for 2015, those associations were not significant.

Positive associations were observed in *Salmonella* spp. between consumption of tetracyclines in animals and resistance in isolates from human infections in the three years of study, although significance was only reached for 2013.

Table 37: Results of logistic regression for consumption of tetracyclines in food-producing animals, expressed in mg/kg of estimated biomass/year, and probability of resistance to tetracyclines in bacteria causing infections in humans

Year	Countries included in the analysis	OR[a]	95% PL CI	p-value
C. jejuni				
2013	AT, DK, EE, ES, IT, LU, NL, NO, SI, SK, UK (n = 11)	1.22	1.14–1.31	< 0.001
2014	AT, EE, ES, FR, IT, LT, LU, NL, NO, PT, RO, SI, SK (n = 13)	1.17	1.09–1.27	< 0.001
2015	AT, CY, DK, EE, FI, FR, IT, NL, NO, PT, RO, SI, UK (n = 13)	1.05	0.96–1.16	> 0.300
C. coli				
2013	AT, ES, IT, LU, NL, SI, SK (n = 7)	1.23	1.07–1.49	< 0.002
2014	AT, ES, FR, LU, NL, PT, SI, SK (n = 8)	1.21	1.06–1.46	< 0.004
2015	AT, CY, EE, FI, FR, IT, NL, PT, RO, SI (n = 10)	1.09	0.96–1.30	> 0.189
Salmonella spp.				
2013	AT, BE, DK, EE, ES, FI, FR, HU, IE, IT, LT, LU, NL, NO, SI, SK, UK (n = 17)	1.14	1.08–1.20	< 0.001
2014	AT, BE, DE, DK, EE, ES, FI, FR, HU, IE, IT, LT, LU, NL, NO, PT, RO, SI, SK, UK (n = 20)	1.07	0.99–1.15	> 0.058
2015	AT, CY, DE, DK, EE, FI, FR, HU, IE, IT, NL, NO, PT, RO, SI, UK (n = 16)	1.04	0.97–1.12	> 0.271

OR: odds ratio; PL CI: profile likelihood confidence interval.
OR varies from 0 to infinity. When OR equals 1 or CI includes 1, the association is not considered statistically-significant.
(a): OR is estimated for a 10-unit increment.

10.6. Multivariate analysis

Data on resistance to tetracyclines from human infections were obtained for *Salmonella* spp., *S*. Typhimurium, *S*. Enteritidis, *S*. Infantis, monophasic *S*. Typhimurium, *C. coli* and *C. jejuni*. Except for *C. coli* they could be analysed in a multivariate model.

For *Salmonella* spp. multivariate analysis involved only 11 countries for which all data were available. No significant relationship could be assessed between resistance in human isolates and other variables. The relationship between the consumption of tetracyclines by animals and resistance in animals *Salmonella* spp. isolates was significant (Figure 48).

For *C. jejuni*, from data available in 14 countries, the direct effect of resistance in poultry on resistance in human isolates was estimated to be 0.746, whereas the indirect effect of tetracyclines consumption in animals was estimated to be 0.530, mediated through the significant relationship between tetracyclines consumption in animals and resistance in animal isolates. About half only of the variance of *C. jejuni* resistance or resistance rate could be explained (R^2 = 0.503 and 0.557 in animals and humans, respectively) (Figure 49).
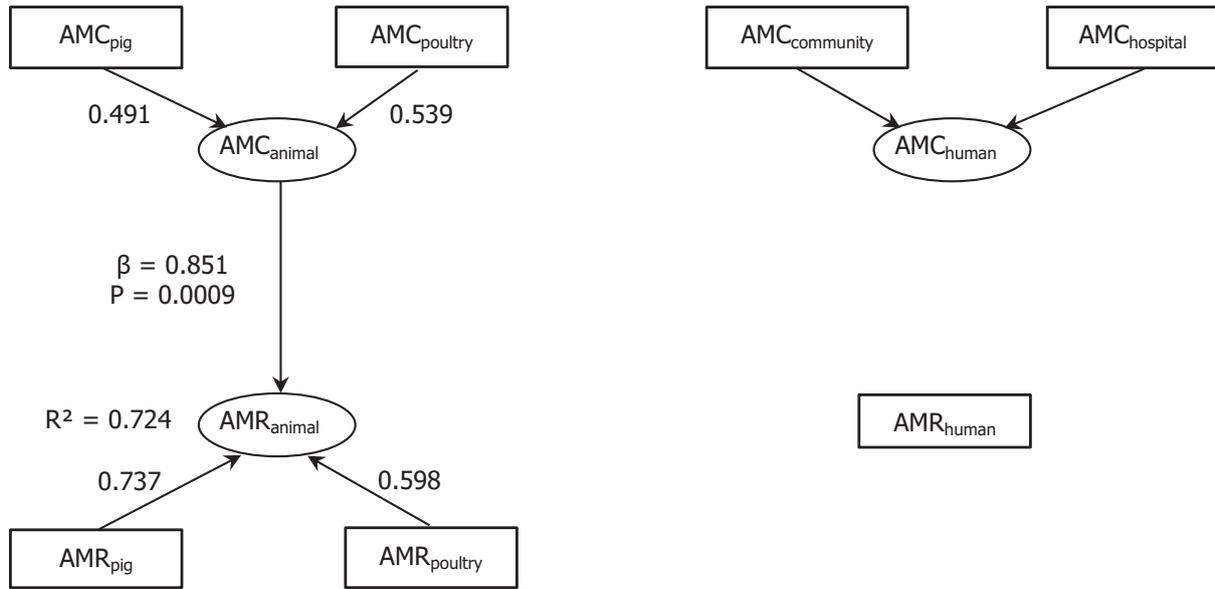
11 countries involved: BE, CY, DE*, DK, ES*†, FR, HU, PT, RO, SK†, UK (goodness-of-fit = 0.736).
†For these countries, estimated consumption in pigs in 2014 was used as a proxy for 2015 missing data.
*For these countries, consumption in hospital was estimated.

Figure 48: Diagram of the PLS-PM model of resistance to tetracyclines in *Salmonella* spp. in humans (in 2014 and 2015) considering resistance to tetracyclines in *Salmonella* spp. from animals (in pigs in 2015 and in poultry in 2014), consumption of tetracyclines in humans (average 2014–2015, expressed in DDD per 1,000 inhabitants and per day), in animals (in pigs in 2015 and in poultry in 2014, expressed in DDDvet/kg of estimated biomass)
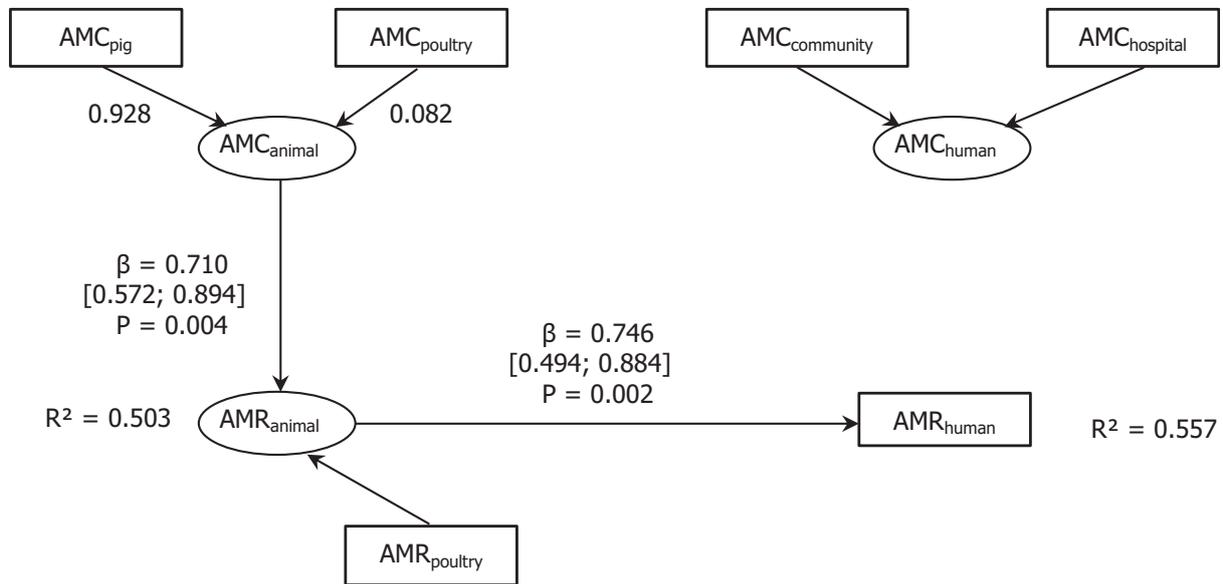
14 countries: AT*, CY, DK, ES*, FI, FR, IT, LT, NL, PT, RO, SI, SK, UK.
*For these countries consumption in hospital was estimated (goodness-of-fit = 0.689).

Figure 49: Diagram of the PLS-PM of resistance to tetracyclines in *Campylobacter jejuni* from humans (2014 and 2015) considering resistance to tetracyclines in *C. jejuni* from animals (poultry 2014), consumption of tetracyclines in humans (2014–2015 average, expressed in DDD per 1,000 inhabitants and per day), in animals (in pigs in 2014 and poultry in 2014 - expressed in DDDvet/kg of estimated biomass)

10.7.   Key findings on tetracyclines

- In most MSs, the amount of tetracyclines consumed in food-producing animals markedly outweighed that consumed in humans. The variation in animal consumption was very wide between countries. No significant correlation was observed within country between consumption of tetracyclines by humans and food-producing animals.
- In humans, the consumption of tetracyclines was, with some exceptions, not correlated with resistance to tetracyclines in *Salmonella* spp. and *Campylobacter* spp. No data were available on invasive *E. coli*.
- In food-producing animals, as well as specifically in pigs and poultry, significant positive associations were generally observed between consumption of and resistance to tetracyclines over the period of study.
- Significant association between rates of resistance to tetracyclines in *Salmonella* spp. from food-producing animals and from humans was found. This correlation was significant using either data at animal species level (broilers) for serovars common in poultry or at aggregate level (SIMR) from the main three food-producing animal species.
- Significant correlations were observed between tetracycline resistance in *C. jejuni* from broilers and turkeys and in humans. Insufficient data was available on non-susceptibility in *C. coli* from pigs and humans to make an analysis which could have shed more light on the relationship between tetracycline resistance in pigs and in humans.
- Significant associations were observed between consumption of tetracyclines in food-producing animals and resistance in *C. jejuni* and *C. coli* in humans.
- In the multivariate analysis on *C.* jejuni, resistance to tetracyclines in bacteria from humans was significantly related to resistance in bacteria from food-producing animals, which, in turn, was significantly related to the consumption of tetracyclines in animals.
- In the multivariate analysis on *Salmonella* spp., resistance to tetracyclines in bacteria from food-producing animals was significantly and positively related to the consumption of tetracyclines in animals. No significant relationships could be assessed between resistance in human isolates and other variables.

11.   Carbapenems

11.1.   Consumption of carbapenems by country

In 2014, the consumption of carbapenems at the hospital constituted 73–100% of the total consumption of carbapenems and in most of the countries (16/23), this percentage was greater than 99%.

In 2014, the mean consumption of carbapenems in humans equalled 0.06 DDD per 1,000 inhabitants per day. The corresponding range was 0–0.19 (median 0.05) DDD per 1,000 inhabitants and per day. The consumption of carbapenems in humans by country in 2014 is shown in Figure 50.

Carbapenems are not authorised for use in animals.
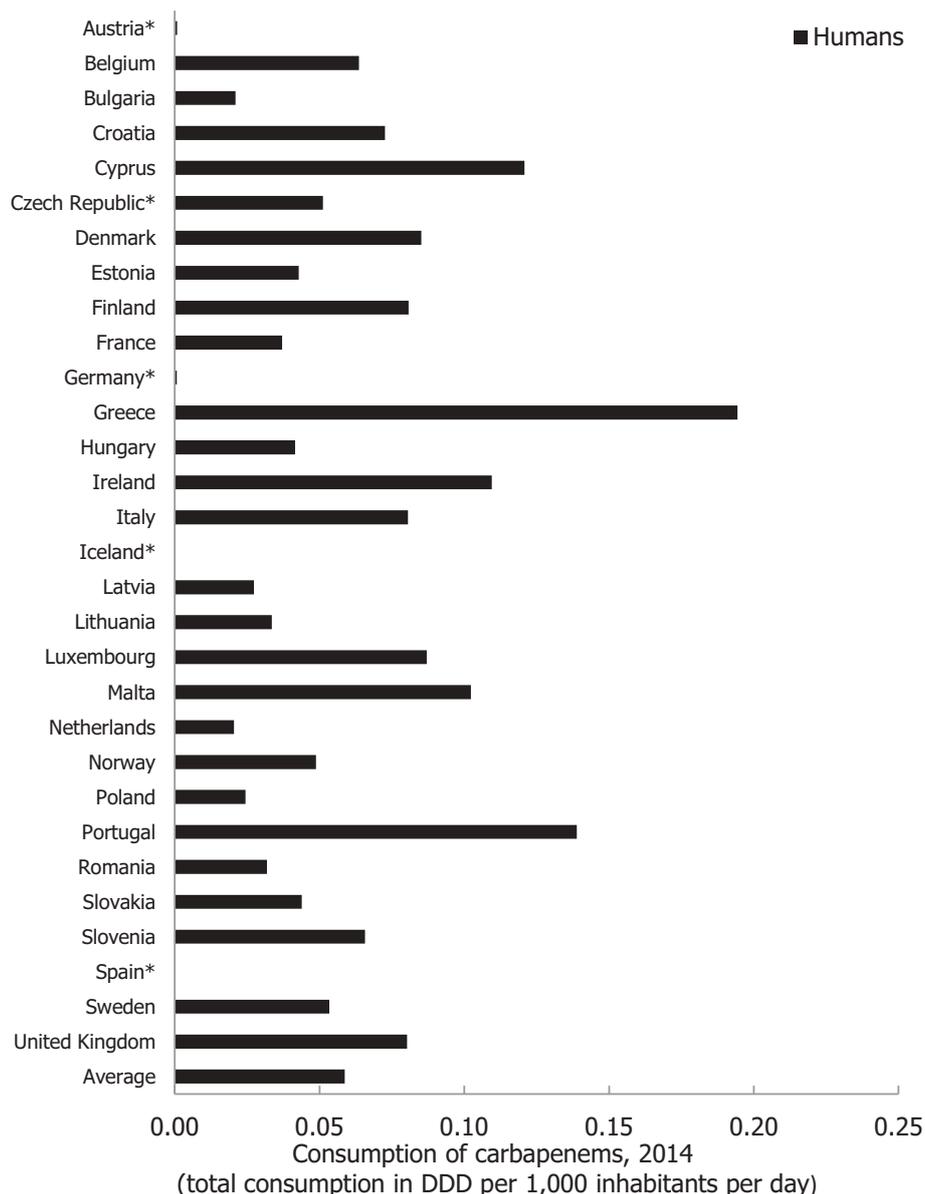
Asterisk (*) denotes that only community consumption was provided for human medicine. The population-weighted mean proportion (%) of the hospital sector from the 2014 total national consumption of antimicrobials for EU/EEA MSs that provided data for both sectors is 94.1%.

Figure 50: Consumption of carbapenems in humans (in the community and at the hospital) expressed in DDD per 1,000 inhabitants and per day, by country, EU/EEA, 2014

11.2. Consumption of carbapenems in humans and occurrence of resistance to carbapenems in invasive *E. coli* and *K. pneumoniae* isolates from humans

The emergence and spread of resistance to carbapenems in Enterobacteriaceae (*Klebsiella pneumoniae* and *E. coli*) has already been documented in all healthcare systems of the EU/EEA MSs. The global rise and subsequent distribution of these resistant bacteria is a public health threat. Carbapenem-resistant Enterobacteriaceae (CRE) and carbapenemase-producing Enterobacteriaceae (CPE) have been mainly associated with healthcare associated infections. The occurrence of infections with community onset by these bacteria has also been documented. In healthcare settings, CRE and especially carbapenemase-producing *K. pneumoniae* isolates have been associated with outbreaks.

For this analysis, susceptibility testing to carbapenems has been performed in invasive *E. coli* and *K. pneumoniae* by use of either meropenem or imipenem, and results interpreted by use of clinical breakpoints. The use of different antimicrobial agents or interpretive criteria may influence the result.

11.2.1. Invasive *E. coli*

In 2013, resistance in invasive *E. coli* isolates to carbapenems was zero in nine countries and < 1% in 19 countries. Two MSs reported 3.4% and 1.9% of invasive *E. coli* resistant to carbapenems, respectively. In 2014, resistance to carbapenems in invasive *E. coli* was zero in eight countries and < 1% in 21 countries (1 MS reported 1.3%). In 2015, resistance in invasive *E. coli* to carbapenems was zero in 11 countries and < 1% in 17 countries. Two MSs had 2.1% and 1.4% of invasive *E. coli* resistant to carbapenems, respectively.

Significant correlation between total (community and hospital) consumption of carbapenems and resistance to carbapenems of invasive *E. coli* isolates was found only for the year 2014 (Table 38, Figure 51). When only hospital consumption of carbapenems was analysed no significant correlations were found with resistance of invasive *E. coli* from humans to carbapenems (data not shown).

Table 38: Results of logistic regression for total (community and hospital) consumption of carbapenems in humans, expressed in DDD per 1,000 inhabitants and per day, and probability of resistance to carbapenems in invasive *E. coli* from humans, EU/EEA, 2013–2015 (see also Figure 51)

Year	Countries included in the analysis	OR	95% PL CI	p-value
Invasive *E. coli*				
2013	AT, BE, BG, CZ, DE, DK, EE, EL, ES, FI, FR, HR, HU, IE, IS, LT, LU, LV, MT, NL, NO, PL, PT, SE, SI, SK, UK (n = 27)	1.07	0.90–1.26	0.426
2014	AT, BE, BG, CZ, DE, DK, EE, EL, ES, FI, FR, HR, HU, IE, IS, LT, LU, LV, MT, NL, NO, PL, PT, SE, SI, SK, UK (n = 27)	1.12	1.04–1.25	0.016
2015	AT, BE, BG, CZ, DE, DK, EE, EL, ES, FI, FR, HR, HU, IE, IS, LT, LU, LV, MT, NL, NO, PL, PT, SE, SI, SK, UK (n = 27)	1.11	0.99–1.26	0.058

OR: odds ratio; PL CI: profile likelihood confidence interval, 95%.
OR varies from 0 to infinity. When OR equals 1 or CI includes 1, the association is not considered statistically-significant.
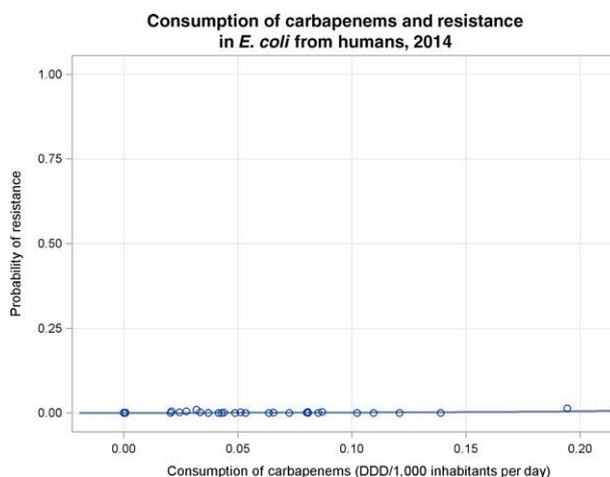
Figure 51: Logistic regression analysis curve of the total (community and hospital) consumption of carbapenems in humans, expressed in DDD per 1,000 inhabitants and per day, and the probability of resistance to carbapenems in invasive *E. coli* from humans, EU/EEA, 2014 (see also Table 38)

11.2.2. Invasive *Klebsiella pneumoniae* isolates

In 2013, resistance to carbapenems in *K. pneumoniae* isolates was 0% in six countries, < 1% in nine countries and > 1% 15 countries. In 2014, resistance to carbapenems in *K. pneumoniae* was 0%

in one country, < 1% in nine countries and > 1% in 20 countries. The corresponding numbers for 2015 was 0% in five countries, < 1% in 11 countries and > 1% in 14 countries.

Strong correlations between resistance to carbapenems and total (community and hospital) consumption of carbapenems were observed for *K. pneumoniae* for all three years (Table 39, Figure 52). Higher total consumption correlated with a higher proportional increase in the occurrence of carbapenem resistance. A higher consumption (by 1 unit DDD) of carbapenems was associated with a higher probability of resistance (25%) in *K. pneumoniae* isolates in 2013. This probability of resistance was 22% in 2014 and 23% in 2015 (Table 39).

Table 39: Results of logistic regression for total (community and hospital) and for hospital consumption of carbapenems in humans, expressed in DDD per 1,000 inhabitants and per day, and the probability of resistance to carbapenems in invasive *K. pneumoniae* from humans, EU/EEA, 2013–2015 (see also Figure 52)

Year	Countries included in the analysis	OR	95% PL CI	p-value
Invasive *K. pneumoniae* – total consumption (community and hospitals)				
2013	AT, BE, BG, CY, CZ, DE, DK, EE, EL, ES, FI, FR, HR, HU, IE, IS, IT, LT, LU, LV, MT, NL, NO, PL, PT, RO, SR, SI, SK, UK (n = 30)	1.25	1.09–1.45	0.002
2014	AT, BE, BG, CY, CZ, DE, DK, EE, EL, ES, FI, FR, HR, HU, IE, IS, IT, LT, LU, LV, MT, NL, NO, PL, PT, RO, SR, SI, SK, UK (n = 30)	1.22	1.06–1.41	0.005
2015	AT, BE, BG, CY, CZ, DE, DK, EE, EL, ES, FI, FR, HR, HU, IE, IS, IT, LT, LU, LV, MT, NL, NO, PL, PT, RO, SR, SI, SK, UK (n = 30)	1.23	1.08–1.42	0.002
Invasive *K. pneumoniae* – hospital consumption				
2013	BE, BG, CY, DK, EE, EL, FI, FR, HR, HU, IE, IS, IT, LT, LU, LV, MT, NL, PT, RO, SE, SI, SK, UK (n = 24)	1.26	1.02–1.57	0.033
2014	BE, BG, CY, DK, EE, EL, FI, FR, HR, HU, IE, IT, LT, LU, LV, MT, NL, PL, PT, RO, SE, SI, SK, UK (n = 24)	1.17	0.94–1.46	0.149
2015	BE, BG, CY, DK, EE, EL, FI, FR, HR, HU, IE, IT, LT, LU, LV, MT, NL, PL, PT, RO, SE, SI, SK, UK (n = 24)	1.25	0.99–1.63	0.058

OR: odds ratio; PL CI: profile likelihood confidence interval, 95%.
Note: OR varies from 0 to infinity. When OR equals 1 or CI includes 1, the association is not considered statistically-significant.
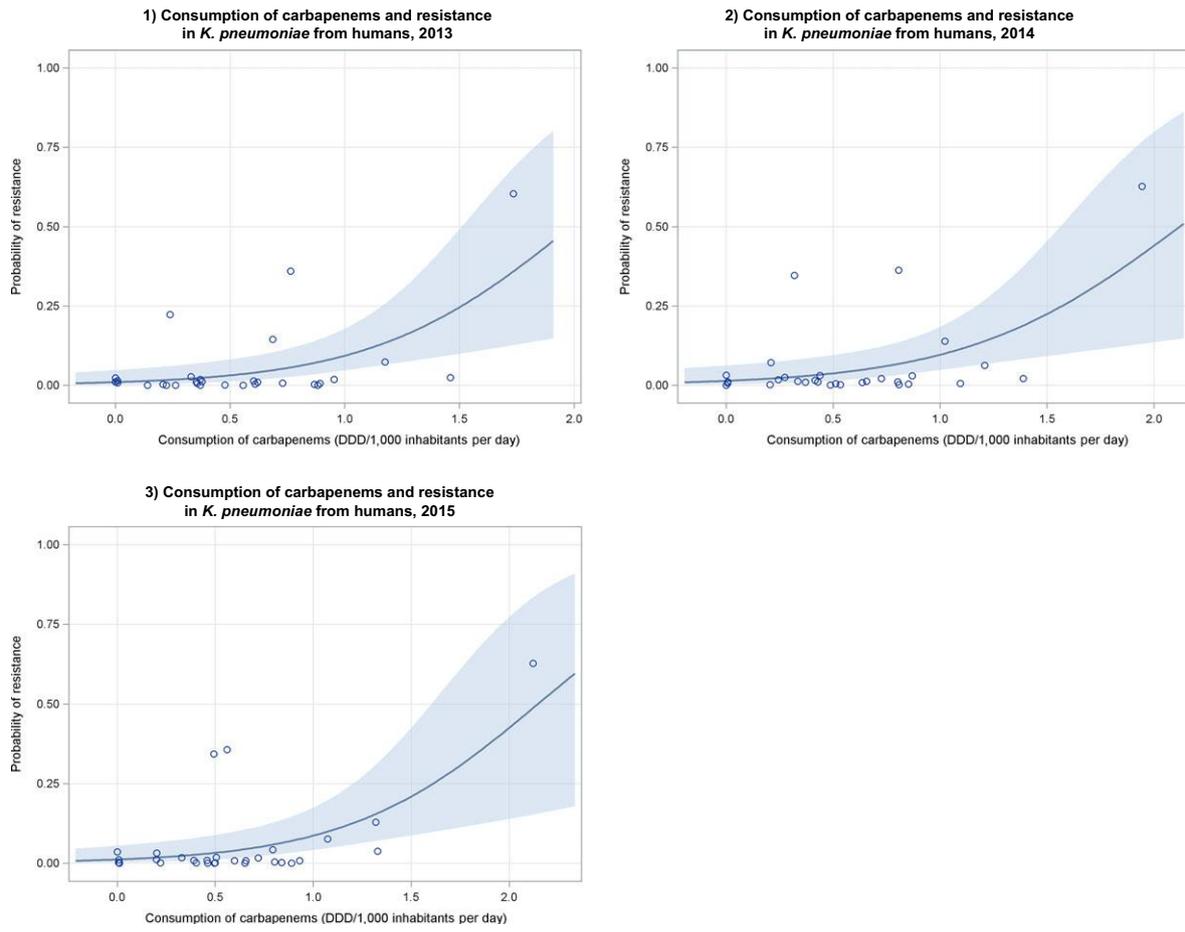
Dots represent countries involved in the analysis.

Figure 52: Logistic regression analysis curves of the total (community and hospital) consumption of carbapenems, expressed in DDD per 1,000 inhabitants and per day, and the probability of resistance to carbapenems in invasive *K. pneumoniae* from humans, EU/EEA, 2013–2015 (see also Table 39)

When the correlation included data only from hospital sector, the consumption of carbapenems was significantly correlated with resistance of *K. pneumoniae* to carbapenems only for 2013 (Table 39).

11.3. Resistance to carbapenems in bacteria from animals *versus* resistance to carbapenems in bacteria from humans

Carbapenems are not approved for use in food-producing animals and *K. pneumoniae* is not considered a zoonotic bacterium. The prevalence of CPE in food-producing animals is not known for 2013–2014. In 2015, resistance to meropenem was tested in both *E. coli* and *Salmonella* spp. from food-producing animals but no resistance was detected. In detail, among 4,268 of indicator *E. coli* isolates from pigs, none was resistant to carbapenems. Among 750 isolates of *Salmonella* spp. from meat from pigs none were resistant to carbapenems. The same was also true for *Salmonella* spp. isolates from fattening pigs (0/424 isolates). For the same year, among 1,734 indicator *E. coli* isolates, none was resistant to carbapenems. Among 80 and 45 *Salmonella* spp. isolates from meat from bovine animals and from calves (under 1 year of age) tested against meropenem resistance was zero.

11.4. Multivariate analysis

Carbapenems are not authorised for use in animals. In addition, surveillance of carbapenem susceptibility of *E. coli* and *Salmonella* spp. in food-producing animals was available in 2015 but detected no resistance. Therefore multivariate analysis for the emergence of carbapenems resistance in *E. coli* or *K. pneumoniae* in relation to the use of carbapenems and resistance to these

antimicrobials in food-producing animals as well as carbapenem use in humans could not be performed.

11.5.   Key findings on carbapenems

- Carbapenems are considered by WHO as CIAs and by AMEG classification as category 3 and are not authorised for use in animals in the EU. Consumption rates of carbapenems in humans, expressed in DDD per 1,000 inhabitants per day, varied among EU/EEA MSs.
- Significant associations were found between the use of carbapenems in humans and resistance to carbapenems in *Klebsiella pneumoniae* from humans for all the three years, while, for invasive *E. coli*, a significant association was only observed in one of the three years under study.
- In food-producing animals, there were only very rare single isolates of *E. coli* or *Salmonella* spp. reported as resistant to carbapenems during the study period and therefore no further analysis could be carried out.

12.   Analysis of possible effect of co-selection

An attempt to study the impact of co-selection on the assessment of the relationship between AMC and AMR in food-producing animals was performed using data on resistance in indicator *E. coli* and the methodology proposed by Søgaard (1989). The analysis addresses SIMR to 3rd- and 4th-generation cephalosporins, fluoroquinolones and tetracyclines in indicator *E. coli* isolates from broilers, pigs and cattle in six countries for the year 2013 and from broilers, turkeys, pigs and veal calves in 10 countries for the period 2014–2015, as well as the corresponding consumption.

12.1.   Resistance to 3rd- and 4th-generation cephalosporins

Considering 2013 data, the use of 'corrected' consumption data resulted in the association becoming significant. Although the point estimates of the ORs decreased while using 'corrected' consumption, the ranges of the CIs were narrowed by the correction performed on the consumption data (Table 40, Figure 53). Considering 2014–2015 data, although the same phenomena of both decreased association and narrowed range of CI were observed when using 'corrected' consumption; the model with 'corrected' consumption was not significant (Table 40). After removing the outlier of the model using 'corrected' consumption (Figure 53); the positive association assessed by the model (OR = 1.000; 95% PL CI: 1.000, 1.001; p-value: 0.022) became significant. Although remaining low, the max-rescaled R-square increases while correcting consumption data on 3rd- and 4th-generation cephalosporins.

Table 40:   Results of logistic regression for consumption and 'corrected' consumption of 3rd- and 4th-generation cephalosporins in food-producing animals, expressed in mg/kg of estimated biomass, and resistance to 3rd- and 4th-generation cephalosporins in indicator *E. coli* from food-producing animals, EU/EEA, 2013–2015 (see also Figure 53)

Year	Consumption	Countries included in the analysis	OR[a]	95% PL CI	p-value
2013	National	AT, BE, CH, DK, ES, HU (n = 6)	1.55	0.99–2.57	0.056
2013	'Corrected'	AT, BE, CH, DK, ES, HU (n = 6)	1.001	1.00[+(b)]–1.002	0.015
2014–2015	National	BE, DE, DK, ES, FR, HR, IT, NO, PT, SE (n = 10)	1.56	1.26–2.01	< 0.001
2014–2015	'Corrected'	BE, DE, DK, ES, FR, HR, IT, NO, PT, SE (n = 10)	1.00	1.00[−]–1.001	0.135

OR: odds ratio; PL CI: profile likelihood confidence interval, 95%.
Note: OR varies from 0 to infinity. When OR equals 1 or CI includes 1, the association is not considered statistically-significant. Regarding resistance data, food-producing animals include broilers, pigs and cattle for 2013 and broilers, turkeys, pigs and veal calves for 2014–2015.
(a): In the absence of 2013 resistance data, proxy data for years prior to 2013 may have been used.
(b): The lower limit of the 95% PL CI is greater than 1.00.
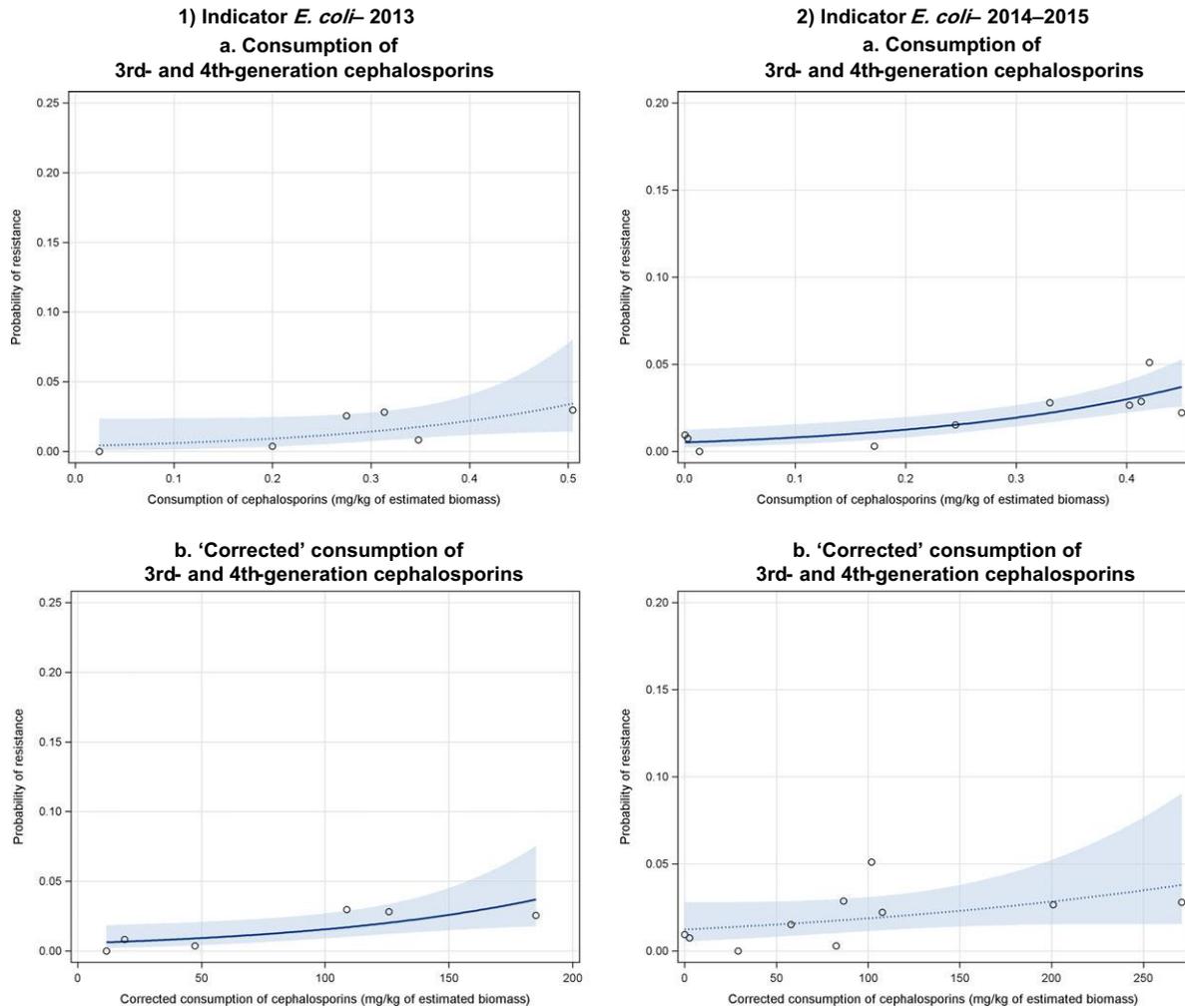
1) Indicator *E. coli*– 2013
a. Consumption of
3rd- and 4th-generation cephalosporins

2) Indicator *E. coli*– 2014–2015
a. Consumption of
3rd- and 4th-generation cephalosporins

b. 'Corrected' consumption of
3rd- and 4th-generation cephalosporins

b. 'Corrected' consumption of
3rd- and 4th-generation cephalosporins

Dots represent the countries included in the analysis. Note: In the absence of 2013 resistance data, proxy data for years prior to 2013 may have been used. Regarding resistance data, food-producing animals include broilers, pigs and cattle for 2013 and broilers, turkeys, pigs and veal calves for 2014–2015. In graphs (1.a). and (2.b.), the dashed curve means that the association is not significant.

Figure 53: Logistic regression analysis curves of the consumption and 'corrected' consumption of 3rd- and 4th-generation cephalosporins in food-producing animals and the corresponding probability of resistance to 3rd- and 4th-generation cephalosporins in indicator *E. coli* from food-producing animals for (1) 2013 and (2) 2014–2015 (see also Table 40)

12.2   Resistance to fluoroquinolones

Considering both 2013 and 2014–2015 data, the use of 'corrected' consumption data resulted in associations of lower strength than those obtained by using the registered consumption and CIs of narrowed ranges. In addition, the sensitivity analysis performed by removing the outlier from the model assessing the relationship between 'corrected' consumption and resistance to fluoroquinolones for the period 2014–2015 showed that the association remained significant and of a similar strength (OR = 1.012; 95% PL CI: 1.01−1.02; p-value: < 0.001). Although remaining low, the max-rescaled R-square increases while correcting consumption data on fluoroquinolones. (Table 41, Figure 54).

Considering specifically SIMR in indicator *E. coli* from food-producing animals in 2013 (Table 41, Figure 54), the association between AMC and AMR for fluoroquinolones was driven by two groups of countries with very different profiles in terms of AMC and AMR (both consumption and resistance low or both high, relative to the spread of values obtained from all countries). The use of 'corrected' consumption resulted in changing the relative situations of the countries regarding AMC and AMR, two

countries exhibiting intermediate situations (both in terms of amounts of consumption and levels of resistance), which better supports the assumed linearity between exposure and risk.

Table 41: Results of logistic regression for consumption and 'corrected' consumption of fluoroquinolones in food-producing animals, expressed in mg/kg of estimated biomass, and resistance to fluoroquinolones in indicator *E. coli* from food-producing animals (see also Figure 54)

Year	Consumption	Countries included in the analysis	OR	95% PL CI	p-value
2013[a]	National	AT, BE, CH, DK, ES, HU (n = 6)	1.19	1.06–1.34	0.003
2013[a]	'Corrected'	AT, BE, CH, DK, ES, HU (n = 6)	1.013	1.007–1.02	< 0.001
2014–2015	National	BE, DE, DK, ES, FR, HR, IT, NO, PT, SE (n = 10)	1.26	1.15–1.40	< 0.001
2014–2015	'Corrected'	BE, DE, DK, ES, FR, HR, IT, NO, PT, SE (n = 10)	1.019	1.005–1.02	0.001

OR: odds ratio; PL CI: profile likelihood confidence interval, 95%.
Note: OR varies from 0 to infinity. When OR equals 1 or CI includes 1, the association is not considered statistically-significant. Regarding resistance data, food-producing animals include broilers, pigs and cattle for 2013 and broilers, turkeys, pigs and veal calves for 2014–2015.
(a): In the absence of 2013 resistance data, proxy data for years prior to 2013 may have been used.

1) Indicator *E. coli*– 2013
a. Consumption of fluoroquinolones

2) Indicator *E. coli*– 2014–2015
a. Consumption of fluoroquinolones

b. 'Corrected' consumption of fluoroquinolones

b. 'Corrected' consumption of fluoroquinolones

Dots represent the countries included in the analysis.
Note: In the absence of 2013 resistance data, proxy data for years prior to 2013 may have been used. Regarding resistance data, foodhyphen;producing animals include broilers, pigs and cattle for 2013 and broilers, turkeys, pigs and veal calves for 2014–2015.

Figure 54: Logistic regression analysis curves of the consumption and 'corrected' consumption of fluoroquinolones in food-producing animals and the corresponding probability of resistance to fluoroquinolones in indicator *E. coli* from food-producing animals for (1) 2013 and (2) 2014–2015 (see also Table 41)

12.3.   Resistance to tetracyclines

Considering 2013 data, the use of the 'corrected' consumption resulted in the association becoming significant. Although the point estimates of the ORs remain of the same magnitude, the range of the CI was narrowed by correction performed on the consumption data. Considering 2014–2015 data, although the use of 'corrected' consumption lowered the strength of the association between, the range of the CI was narrowed. Although remaining low, the max-rescaled R-square increases while correcting consumption data on tetracyclines. (Table 42, Figure 55).

Table 42:   Results of logistic regression for consumption and 'corrected' consumption of tetracyclines in food-producing animals, expressed in mg/kg of estimated biomass, and resistance to tetracyclines in indicator *E. coli* from food-producing animals (see also Figure 55)

Year	Consumption	Countries included in the analysis	OR	95% PL CI	p-value
2013[a]	National	AT, BE, CH, DK, ES, HU (n = 6)	1.010	1.000–1.021	0.049
2013[a]	'Corrected'	AT, BE, CH, DK, ES, HU (n = 6)	1.009	1.004–1.015	0.001
2014–2015	National	BE, DE, DK, ES, FR, HR, IT, NO, PT, SE (n = 10)	1.027	1.015–1.043	< 0.001
2014–2015	'Corrected'	BE, DE, DK, ES, FR, HR, IT, NO, PT, SE (n = 10)	1.013	1.006–1.021	< 0.001

OR: odds ratio; PL CI: profile likelihood confidence interval, 95%.
Note: OR varies from 0 to infinity. When OR equals 1 or CI includes 1, the association is not considered statistically-significant. Regarding resistance data, food-producing animals include broilers, pigs and cattle for 2013 and broilers, turkeys, pigs and veal calves for 2014–2015.
(a): In the absence of 2013 resistance data, proxy data for years prior to 2013 may have been used.

1) Indicator *E. coli* – 2013
a. Consumption of tetracyclines

2) Indicator *E. coli* – 2014–2015
a. Consumption of tetracyclines

b. 'Corrected' consumption of tetracyclines

b. 'Corrected' consumption of tetracyclines

Dots represent the countries included in the analysis.

Note: In the absence of 2013 resistance data, proxy data for years prior to 2013 may have been used. Regarding resistance data, food-producing animals include broilers, pigs and cattle for 2013 and broilers, turkeys, pigs and veal calves for 2014–2015.

Figure 55: Logistic regression analysis curves of the consumption and 'corrected' consumption of tetracyclines in food-producing animals and the corresponding probability of resistance to tetracyclines in indicator *E. coli* isolates from food-producing animals for (1) 2013 and (2) 2014–2015 (see also Table 42)

12.4. Key findings on possible effect of co-selection

- The use of 'corrected' consumption data (accounting for co-selection) for specific antimicrobial classes used in food-producing animals in assessing the relationship between consumption and resistance in indicator *E. coli* resulted in the association becoming statistically-significant for 3rd- and 4th-generation cephalosporins in 2013 and tetracyclines in 2013.
- In the case of all three antimicrobial classes examined, the use of 'corrected' consumption data resulted in narrowing of the confidence interval of the odds ratio. A decrease in the strength of association taking into account 'corrected' consumption data was noted for 3rd- and 4th-generation cephalosporins and fluoroquinolones, whereas for tetracyclines the strength of the association remained approximately the same.

13. Total antimicrobial consumption in relation to complete susceptibility in indicator *E. coli* from food-producing animals

In order to investigate the possible relationship between overall AMC and complete susceptibility in commensal bacteria in food-producing animals, the occurrence of complete susceptibility to the common set of antimicrobials tested for commensal indicator *E. coli* isolates from food-producing animals was compared with the total AMC in animals (macrolide consumption excepted) (expressed in mg per kg of estimated biomass) for the years 2013 and 2014–2015, considered together, at the country level. The category 'food-producing animals' included broilers, pigs and cattle for 2013 and broilers, turkeys, pigs and calves for the period 2014–2015. Macrolide consumption was disregarded from the total AMC, as *E. coli* is naturally resistant to most macrolides. Both data on complete susceptibility and overall AMC were available together for 9 and 24 countries in 2013 and 2014–2015, respectively.

Marked variations in the levels of complete susceptibility and the overall AMC were observed among the countries involved in the analysis (Figure 56) in 2013 and 2014–2015. Complete susceptibility ranged between 80% in some countries to very low or zero levels. Total AMC varied from a few tens of mg/kg of estimated biomass to 300 or 400 mg/kg of estimated biomass in 2013 and 2014–2015, respectively.

For both 2013 and 2014–2015, significant negative associations of the same magnitude were observed between the probability of complete susceptibility and the overall consumption of antimicrobials in animals (Table 43). Considering the data for 2014–2015, a sensitivity analysis shows that the association remains significantly negative after ignoring the three points displayed in the upper left corner of the graph ($p < 0.0001$, $OR_{\text{10-unit increment}} = 0.95$, 95% PL CI: 0.92–0.97, 21 countries). A higher total AMC of 10 mg/kg of estimated biomass decreased the chance of picking up a completely susceptible (to the common set of antimicrobials tested) commensal indicator *E. coli* from the intestinal tracts of the food-producing animals by nearly 10% in both 2013 and 2014–2015 analyses.

Table 43: Results of logistic regression for total national AMC in food-producing animals, expressed in mg/kg of estimated biomass, and complete susceptibility to the harmonised set of substances tested in indicator *E. coli* from food-producing animals (see also Figure 56)

Bacteria	Year	Countries included in the analysis	OR[(a),(b)]	95% PL CI	p-value
Indicator *E. coli*	2013	AT, BE, CH, DE, DK, ES, FI, HU, SE (n = 9)	0.91	0.87–0.95	< 0.001
Indicator *E. coli*	2014–2015	AT, BE, CY, CZ, DE, DK, EE, ES, FI, FR, HR, HU, IE, IT, LT, LV, NL, NO, PL, PT, SE, SI, SK, UK (n = 24)	0.92	0.88–0.95	< 0.001

OR: odds ratio; PL CI: profile likelihood confidence interval, 95%.
Note: OR varies from 0 to infinity. When OR equals 1 or CI includes 1, the association is not considered statistically-significant. Regarding resistance data, food-producing animals include broilers, pigs and cattle for 2013 and broilers, turkeys, pigs and veal calves for the period 2014–2015.
(a): OR is estimated for 10-unit increment.
(b): In the absence of 2013 resistance data, proxy data for years prior to 2013 may have been used.

1) Probability of complete susceptibility – 2013

2) Probability of complete susceptibility – 2014–2015
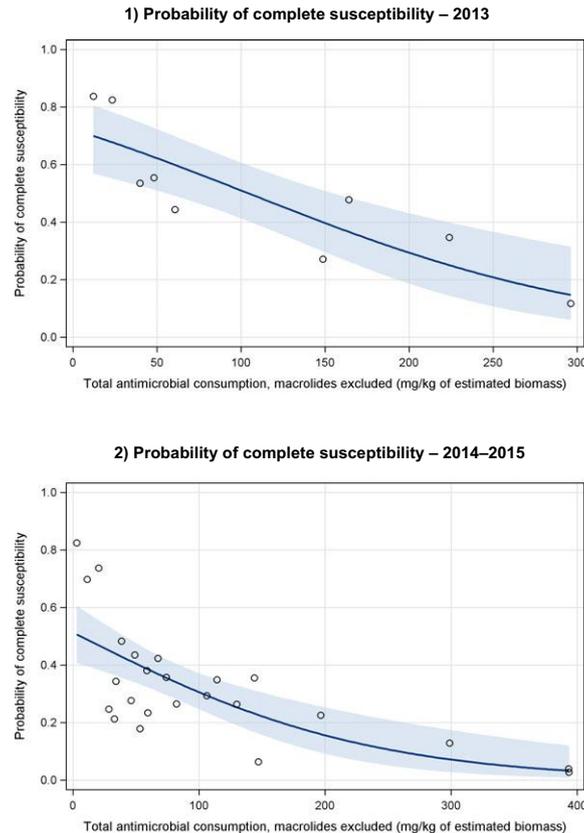
Dots represent the countries included in the analysis.

Regarding resistance data, the category 'food-producing animals' includes broilers, pigs and cattle for 2013, and broilers, turkeys, pigs and veal calves for 2014–2015.

Figure 56: Logistic regression analysis curves of the total national consumption of antimicrobials in food-producing animals and (1) the probability of complete susceptibility to the harmonised set of substances tested in indicator *E. coli* isolates from food-producing animals for 2013 and (2) the probability of complete susceptibility to the harmonised set of substances tested in indicator *E. coli* isolates from food-producing animals for 2014–2015 (see also Table 43)

13.1. Key findings on total AMC in relation to the proportion of complete susceptibility in indicator *E. coli* isolates in food-producing animals

- Complete susceptibility, in the context of this report and in the analysis which was performed, refers to susceptibility to each of the substances of the standard panel of antimicrobials tested. The analysis was possible for indicator *E. coli* from food-producing animals, where a standard panel of antimicrobials was tested, but not for *E. coli* from humans.
- A statistically-significant negative association between higher consumption of antimicrobials and the occurrence of completely susceptible indicator *E. coli* was observed in data for 2013 and in the combined data for 2014 and 2015, with a clear and consistent reduction in the probability of detecting complete-susceptible indicator *E. coli* when more antimicrobials were consumed.

14.   General discussion

14.1.   Inherent characteristics of analysed data

The data analysed in this report were obtained from a number of EU initiatives and networks. These data were collected for purposes other than the main objective of this study, which was to investigate potential relationships between AMC and AMR. The level of granularity[4] of the data in this type of AMC/AMR analysis may impact on the results obtained. Refining the target population and collecting more accurate data might reveal associations which were not apparent with in data collected at the national level. These analyses have detected a number of statistically-significant associations using data relating to the national level (ecological analysis) and provided an overview of the general situation, which, although subject to the various caveats and provisions which were set out in the first JIACRA report (ECDC/EFSA/EMA, 2015), are nonetheless considered useful in this report to describe the overall impact of AMC in humans and animals on AMR in bacteria from humans and animals.

14.1.1.   Uncertainty and imprecision of measurements

14.1.1.1. AMC data in humans and animals

Although based on the best existing data currently available for the EU/EEA, AMC expressed in mg per kg of estimated biomass and per year for both information and for comparison purposes between humans and food-producing animals, is a crude indicator that must be interpreted with caution. The indicator used, i.e. milligrams per kilogram of estimated biomass, does not account for differences in dosing between antimicrobial substances and between humans and food-producing animals. To improve this, an agreed and truly comparable unit of measurement would be needed. More complete AMC data at the animal species level are also necessary for more meaningful comparisons.

Furthermore, the denominator used for AMC in humans may be an overestimate, as data on the weights of humans are uncertain and the ages at risk for treatment (children and elderly being more frequently treated than other age groups) were not taken into account (Blix et al., 2007). For AMC in animals, the denominator is a sum of the mass of different animal species and does not account for differences in the relative composition of the summed national animal populations, as AMC may differ markedly between the various animal populations (i.e. production sectors) as well as by age category (age at risk) of a given animal species. Nevertheless, there is a good correlation between AMC in humans expressed in DDD per 1,000 inhabitants and per day and in mg per kg of estimated biomass and per year, both at the overall level or for each antimicrobial class (ECDC/EFSA/EMA, 2015).

It is estimated that, in all EU/EEA MSs, overall AMC in the community represents, on average, 90% of the total (community and hospital sector data) AMC in humans. In a few countries only reporting community AMC in humans, total AMC (expressed as mg per kg of biomass) in humans was slightly underestimated. However, when interpolating the missing hospital sector data for these countries to the average proportion of 10% of the total national consumption, the EU/EEA median and the population-adjusted average (expressed as mg per kg of biomass) increased only marginally by less than 3%. Thus, the missing data did not influence any significant difference of the overall AMC at the EU/EEA level. This confirms the conclusion, that, on average, more antimicrobials were consumed in food-producing animals than in humans, when expressed in mg/kg biomass.

The average proportion of the hospital sector consumption out of the total national consumption differs when considering specific antimicrobial classes or subclasses. For example, carbapenems are nearly exclusively used in the hospital sector, whereas the vast majority of fluoroquinolones is consumed in the community. There are significant differences in AMC between animals and humans for each antimicrobial class at EU/EAA level. Adding interpolated missing hospital sector data for antimicrobial classes to the human consumption would not significantly change the existing differences between AMC in animals and humans (expressed in mg/kg biomass). For example, this would be the case for comparison of polymyxins consumption, where the vast majority of polymyxins is consumed in food-producing animals or for consumption of 3rd- and 4th-generation cephalosporins, where the vast majority is consumed in humans (please see also Figure 7).

In addition, data coverage of community AMC in humans was not 100 % in all included countries. The countries with less than 95 % data coverage of community AMC were Germany (85 %) and the

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[4] The term granularity reflects here whether the AMC and AMR data relate to an individual farm or hospital, or a region or a country and how representative the measurements of AMC and AMR are in relation to the corresponding population.

Netherlands (92 %). In the latter countries, AMC expressed in tonnes (prior to correction for population or biomass) was underestimated. Still, this underestimate represented less than 5% of the overall total AMC in humans in the included EU/EEA MSs when expressed in tonnes.

Other limitations that may hamper the comparison of AMC in humans and in food-producing animals were discussed in the first JIACRA report (ECDC/EFSA/EMA, 2015).

14.1.1.2. Antimicrobial resistance data

In food-producing animals, the representative nature of the sampling performed and the adoption of identical AST methodologies facilitated the investigation of associations between AMC and AMR in a standardised way in different reporting countries. Nevertheless, the aspects relating to the degree to which consumption can be linked to particular species or production types of animals or classes of human patients, the occurrence of genetic linkage of AMR genes and the issue of cross-resistance between some or all antimicrobials within an antimicrobial class are all factors that increase the complexity of this type of analysis. Consequently, the analysis did not attempt to evaluate AMC and AMR in food-producing animals and humans for all available combinations of antimicrobials and bacterial organisms but conversely focussed on certain combinations of interest.

In determining those combinations and sources of bacteria which were addressed in the report, consideration was given to the amount of data available. The extent to which AMC and AMR data were available was variable, particularly where data from MSs were submitted on a voluntary basis. Available data on AMR in bacteria from meat were disregarded in the context of this report, as they were considered insufficient to perform meaningful analyses. Unlike bacterial isolates sampled from the intestinal flora of healthy animals at slaughter, the bacteria present on meat may be influenced by production processes which influence bacterial survival. Furthermore, the sources of bacteria on meat include not only the animals from which the meat was derived, but also the persons involved in meat production as well as the environment in which meat was prepared or stored. Cross-contamination between meat originating from different national sources might also occur. Meat samples may represent domestic production and/or meat which may have been imported; distinguishing between these sources is essential for a meaningful analysis of AMR in bacteria from meat in relation to AMC in animals, because differences in exposure to antimicrobials may occur in different countries.

The comparisons between AMR in relevant bacteria from food-producing animals and humans may be hampered by the difference of sampling in the two sectors, which mainly originate from the different objectives of the monitoring networks. For humans, AMR data were based on information from clinical isolates, for which resistance is determined using clinical breakpoints primarily to guide treatment. For this report, this was particularly applicable to invasive *Klebsiella pneumoniae* and *E. coli* isolates from humans. For *Salmonella* spp. and *Campylobacter* spp., AST results are interpreted using ECOFFs, or aligned closely with the ECOFF for already interpreted data. In food-producing animals, a unified sampling scheme based on the EU legislation and the interpretation of resistance using ECOFFs, were applied. Thus, results for *Salmonella* spp. and *Campylobacter* spp. were, in general, directly comparable between both sectors, whereas findings on *E. coli* were less so, with a difference of one to four dilution steps, depending on the antimicrobial, between the sectors (see Figure 4). Furthermore, the overall comparison of resistance in *E. coli* from humans and food-producing animals is difficult because of the different populations under surveillance – i.e. invasive *E. coli* isolates from human BSI vs. primarily non-pathogenic indicator commensal *E. coli* isolates from healthy animals. Without characterisation of the bacteria from the two populations involving both 'traditional' methods (e.g. serotyping) and molecular characterisation of, for example, resistance genes, any apparent associations between the two populations regarding AMR are difficult to assess.

Between half and two-thirds (varies by year) of the data from human *Salmonella* spp. and *Campylobacter* spp. isolates are from the national public health reference laboratories (NPHRLs) and may not always be a representative selection of all *Salmonella* spp. and *Campylobacter* spp. infections in the countries even though certain countries test all isolates at the central reference laboratory level. Most importantly, several countries do not routinely test all isolates of *S.* Enteritidis for AMR, since, in the EU, most isolates of this serovar are in general susceptible. *S.* Enteritidis is the most common serovar in human infections and when excluded from the AMR results, the resistance for *Salmonella* spp. will subsequently be overestimated. For surveillance or research purposes, some countries have focussed on testing specific serovars with high-level fluoroquinolone resistance or MDR (e.g. *S.* Kentucky, monophasic *S.* Typhimurium). Due to the role of the NPHRLs, there is also a risk of over-representation of isolates that are difficult to type or strains from outbreaks (though of less impact for *Campylobacter* spp. where outbreaks are rare or more difficult to detect than for *Salmonella* spp.).

Furthermore, the development of the collection of data over time, in particular regarding the comparison of AMR in food-producing animals, between the first and the second JIACRA report must be considered. In the first JIACRA report, the definition of poultry in 2013 was that of 'broilers', whereas for 2014 (the second JIACRA report) poultry encompassed both 'broilers and turkeys'. Similarly in 2013, bovine animals were categorised as 'cattle', whereas in 2015, AST in bovine animals was confined to veal calves. Thus, the SIMR is not exactly the same for the above species between 2013 and 2014–2015.

Lastly, in order to include as many data points as possible, particularly for the pathogenic zoonotic bacteria, a minimum of ten isolates tested for the bacteria and drug combination per country and year was set. Such a low cut-off is sensitive to random variation, which may have resulted in large variations in the proportion of non-susceptible isolates.

14.1.2. Ecological data and ecological analysis

This second JIACRA report constitutes an attempt to provide an integrated multivariate ecological study of available data on AMC and AMR in bacteria from humans and food-producing animals, provided by the EU-wide surveillance/monitoring programs. The report investigated the occurrence of AMR in selected pathogenic and non-pathogenic bacteria in 2013, 2014 and 2015 in both the human and animal sectors in relation to the consumption of antimicrobials in these sectors, and also using data generated to investigate possible links between AMC in the food-producing animals and AMR in humans. The potential direct relationships between AMC in humans and AMR in animals were not addressed in this report. Investigation of this latter relationship would ideally require data relating to a bacterial organism occurring in humans where the bacterium is exposed to AMC, with the additional condition that this bacterium is relatively frequently acquired by animals from humans. Since such data are not currently available, this analysis was not performed.

Ecological analyses can be used to investigate in an exploratory manner the impact of risk-modifying factors on health/non-health outcomes based on populations defined either geographically or temporally. Both risk-modifying factors and outcomes were considered at the population level in each geographical unit and then compared, and their potential association assessed using standard statistical methods. For example, AMC and AMR in a given population -whether animal or human- were compared across a number of countries. Although ecological studies are particularly useful for generating hypotheses since they can use existing datasets, classically derived from monitoring/ surveillance programs, they are limited by the fact that they cannot look at cause and effect in individuals and therefore cannot establish causation, no matter how strong the associations discerned. The findings of ecological analyses, such as those presented in the second JIACRA report, are not causal assessments. As traditional criteria for causality were not met, it cannot be excluded that concomitant phenomena were observed without any causal relationship. It is important to take this into account when interpreting the results of the analyses presented in this report.

Within the framework of the ecological analyses, small datasets including up to a maximum of about 30 countries -statistical units of the ecological analyses were countries- were used. Limited numbers of outliers (outlying countries) of two types - either low AMC and high AMR or high AMC and low AMR- were commonly observed throughout the analyses. These might have significant impact on the results, as logistic regression may be sensitive to outliers. Sensitivity analyses were performed when deemed appropriate. The distribution of countries in graphs of consumption vs. resistance do not provide details of the epidemiology or underlying reasons for observed differences, but they provide a starting point which might be useful as a tool in stimulating relevant investigations. For example, in the animal sector, while analysing the possible relationship between AMC and AMR to fluoroquinolones in *E. coli/Salmonella*, such outliers, exhibiting low consumption of fluoroquinolones but high resistance to these substances in the years 2013 and 2014–2015, were observed. Such situations may reflect historical use, or the diffusion of multi-resistant clones co-selected by the use of other classes of antimicrobials. Such diffusion may be facilitated by the pyramidal structure of certain animal production sectors. In this respect, the widespread distribution in the poultry sector of bacteria resistant to 3rd-generation cephalosporins in the early 2000s has been suggested to be related to this pyramidal structure (Liebana et al., 2013).

14.2.   Inherent characteristics of the statistical methods used

14.2.1.   Logistic regression

Logistic regression was used to assess the relationships between the different available sets of data as shown in Figure 1 – with the only exception of assessing any relationship between AMC in animals and humans, which was assessed using Spearman's rank correlation test. The assumption was made that the independent variable (e.g. AMC) is linearly related to the log-odds of the dependant variable (e.g. AMR), i.e. the OR represents the average OR for each incremental unit (expressed in either DDD or mg per kg of estimated biomass) of AMC over the entire scale. Homogeneity of the OR through the AMC scale was not checked considering the low number of observations and therefore, heterogeneity of the association among the AMC scale cannot be excluded.

14.2.2.   Partial Least Squares Path Modeling (PLS-PM)

The datasets analysed in the univariate and the multivariate analysis were not exactly the same. The multivariate analysis included data on AMR in bacteria from animals for 2014 and 2015, and corresponding pooled or averaged data on AMR in bacteria from humans, involving, where necessary, proxy or estimated data in order to limit the exclusion of certain observations (i.e. countries) due to missing data. Conversely to the results of the univariate analyses, the results of the multivariate models cannot be expressed as odds ratios, since PLS-PM is based on a linear assessment of the relationship between the latent variables.

PLS-PM was elected to perform multivariate analyses, as it allows presenting and accounting for the biological knowledge of the complex relationships between AMC and AMR data, as represented in Figure 1. For AMR in bacteria from humans, AMC in animals could be considered as either a direct or an indirect independent variable –in the latter case, through impact on AMR in bacteria from food-producing animals, possibly subsequently transmitted to humans. PLS-PM is also particularly suited when there is multicollinearity between independent variables and when observations are few compared with the number of independent variables. Although PLS-PM does not impose sample size assumption, a minimum of twenty observations are frequently mentioned in the literature as required for PLS-PM. Within the framework of this report, considering the strength of the relationships, the small number of independent variables and the low complexity of the network of relationships, some models were still computed by including between 10 and 20 observations. In such cases, no bootstrap was performed to estimate confidence intervals. The limited number of observations should be kept in mind while interpreting the results of multivariate analysis.

The multivariate analysis should be considered as both structural representation and assessment of the relationships that could be explored between all data available, still summarizing data components (data measured in different animal species or in different settings) through latent variables. The multivariate models determined both the significance and the magnitude of the relationships between AMR in bacteria from humans and i) AMC in humans (as a combination of the consumptions in the community and at the hospital), ii) AMR in bacteria from animals (as a combination of AMR data on pigs and poultry), while considering the impact of AMC in animals (as a combination of AMC data in pigs and poultry). All the potential relationships were not addressed independently in a two by two assessment at the food-producing animal/species level as in the univariate analysis, but in a simultaneous manner. As a consequence, some relationships, even though found statistically-significant in the univariate analysis, did not remain in the final multivariate model, such as the relationship between fluoroquinolone resistance in *E. coli* from animals and fluoroquinolone resistance in *E. coli* from humans. This was not inherently due to the absence of a relationship but to the strong association and impact of the consumption of fluoroquinolones and other quinolones in humans. A large part of the variation in the dependent variable was thus explained, and the remaining part could not be significantly explained by AMR in bacteria from animals, considering the set of observations offered to the model.

The PLS-PM models assessed the relationships between AMR in bacteria from humans and corresponding AMC and AMR in bacteria from pigs and poultry only (and AMR in bacteria from poultry only for *C. jejuni* models). As AMC and AMR in other animal species, as well as AMR in other reservoirs could also play a role, the results of PLS-PM models were an attempt to estimate the relative influence of the parameters addressed in the analysis, and therefore should not be interpreted as a comprehensive overview of the determinants of AMR in bacteria from humans.

14.3.   Interpretation and discussion of results

Comparison of antimicrobial consumption

Compared to the first JIACRA report, the number of reporting countries increased for both AMC data in humans and in animals. Data on AMC in both humans and food-producing animals were available from 28 countries in 2014 compared with 26 countries in 2012. For the human sector, hospital AMC data were not available in nearly 18% of the countries included in the analysis. When analysing for correlation of human consumption and AMR or within MVA, a sensitivity analysis or interpolation of missing hospital sector data was performed to consider this limitation. In most cases, the outcome when considering the missing data did not change significantly, which strengthened the validity of AMC data from humans despite missing hospital AMC data for a few countries.

When using the jointly applied indicator for AMC expressed in milligrams per kilogram of estimated biomass and per year, in two thirds of countries reporting AMC data in both sectors, overall AMC was lower in food-producing animals than in humans. In nearly one third of the countries, AMC in humans was lower than in food-producing animals. Still, the population weighted average was higher for AMC in animals than in humans for the countries included in the analysis.

For four of the antimicrobial classes studied, restrictions on consumption in animals are recommended. The 3rd- and 4th-generation cephalosporins, fluoroquinolones and other quinolones, and polymyxins are all placed in category 2 of the AMEG list, indicating that restrictions on use of these substances in animals are needed (EMA/AMEG, 2016). For macrolides and tetracyclines, prudent use should also apply. Carbapenems are not authorised for use in animals in the EU. Consumption of polymyxins in food-producing animals by far outweighed consumption in humans. In 2016, AMEG has advised that the consumption of polymyxins in food-producing animals should be reduced to the lowest possible level (EMA/AMEG, 2016).

The AMC data presented, whether considering figures of overall consumption or consumption of selected antimicrobial classes or subclasses, showed marked variations between countries in both animal and human sectors. This indicates that there is still a potential for improving appropriate use in both sectors. In the animal sector, the potential of such improvements may differ by country if the burden of bacterial disease requiring antimicrobial treatment is significantly different between countries. However, some countries with moderate or higher AMC have taken initiatives and are reporting progress in national reports. Thus the situation may have changed since 2014. Some countries have proven that it is possible to effectively reduce AMC in animals by applying a set of measures, including setting targets for reduction of AMC (EMA/EFSA, 2017).

Occurrence of the target bacterium

The analyses performed in this report used all available data from the EU MSs and other reporting countries, although the associations detected for *E. coli,* as well as for *C. jejuni* and related antimicrobials in poultry, were usually much stronger than for *Salmonella* spp., where statistically-significant associations were much less frequently detected. The prevalence of *Salmonella* spp. varies greatly between countries. In most countries, *Salmonella* spp. is not ubiquitous, whereas *E. coli* is ubiquitous and *C. jejuni* is very common in the species of poultry studied. Thus, there may be countries with high or low AMC in a sector, but a lack of general exposure of the target bacteria because of its limited occurrence. The first JIACRA report commented in detail on these aspects and in particular, the role of clonal spread of resistant bacteria, which is often significant in *Salmonella* spp. The investigation of associations between AMC and AMR for bacteria such as *Salmonella* spp., which are not ubiquitous, probably requires studies at a more detailed level.

Mutational versus acquired, plasmid-mediated resistance

Considering those antimicrobials of public health relevance as first-line treatments for invasive Gram-negative infections in humans, i.e. fluoroquinolones and 3rd- and 4th-generation cephalosporins, differences were observed in the numbers of significant associations detected between the two antimicrobial classes. There are genetic differences in the predominant mechanisms by which bacteria develop resistance to these two agents; for fluoroquinolones, mutational resistance frequently develops (although plasmid-mediated transferable resistance also occurs). For 3rd- and 4th-generation cephalosporins, resistance is usually plasmid-mediated (although promoter mutations of the *E. coli* endogenous ampC enzyme can also occur in *E. coli* but not in *Salmonella* spp., which do not possess an endogenous ampC enzyme). These differences mean that for the fluoroquinolones, no external

source of AMR genes is required for the common mechanism of AMR to occur and consumption of fluoroquinolones will select for mutations when they occur. For plasmid-mediated ESBL or AmpC resistance to 3rd- and 4th-generation cephalosporins, a source of ESBL or AmpC genes is required from bacteria in the environment or from other bacteria which naturally-possess these enzymes on their chromosome. Therefore, the degree of availability of ESBL or AmpC beta-lactamase genes in the environment, which can be acquired by the target bacteria studied in this report, seems likely to be another factor which may influence the results and the associations detected. The degree of availability and ubiquity of resistance mechanisms in the bacterial environment, including the intestinal tract of animals or humans, as well as the external environment (for example in surface water), might therefore also be factors influencing results. This may be of particular relevance for colistin (see below), for which consumption in the absence of a source of the transferable resistance gene *mcr-1*, does not lead to increases in *mcr-1* related resistance, but when the gene is introduced into a system where colistin is used, then bacteria possessing this gene are selected and may spread.

3rd- and 4th-generation cephalosporins

Considering further the inherent characteristics of ecological studies, resistance to 3rd-generation cephalosporins in invasive *E. coli* from humans was found to be mostly related to the human consumption of 3rd- and 4th-generation cephalosporins. As 3rd- and 4th-generation cephalosporins are mostly used in the human sector, such a result was expected. It also reinforces the point that those substances should continue to be used judiciously in human medicine. No association was found between consumption in animals and resistance to 3rd- generation cephalosporins in animals, whether considering indicator *E. coli* or *Salmonella* spp. Consumption of 3rd- and 4th-generation cephalosporins in food-producing animals is generally low. This might be attributed partly to the fact that only products for individual administration are used, but also to recommendations on limiting their use in animals (EMA/CVMP, 2012; EMEA/CVMP, 2009), in particular the recommendation not to use these antimicrobials in poultry, for which no marketing authorisation has been granted. Use of 3rd- and 4th-generation cephalosporins should ideally continue to be low on the animal sector in order to avoid an increase in AMR similar to that recorded in the human sector.

Fluoroquinolones and other quinolones

In some countries, stewardship and prudent use initiatives may be promoted in both sectors, possibly accounting for the correlation found between consumption of fluoroquinolones and other quinolones in humans and food-producing animals at the country level.

For *E. coli*, the multivariate analysis found significantly positive associations between consumption of fluoroquinolones in each sector and resistance in bacteria from each sector. Strong associations between fluoroquinolone consumption and resistance in *E. coli* have been described previously in national (Gallini et al., 2010; Jensen et al., 2010) as well as international (Durham et al., 2010; ECDC/EFSA/EMA, 2015) ecological studies and are in line with the findings of this study. In addition, the association detected between the consumption of fluoroquinolones in the community and the resistance of invasive *E. coli* from BSI in hospitals might be explained by the analyses performed on EARS-Net data, which have shown that *E. coli* is the bacterium with the highest proportion of community associated bloodstream infections among the Gram-negative bacteria reported to EARS-Net (Heuer et al., 2014).

According to the univariate analyses for *E. coli*, statistically-significant positive associations were also found between the consumption of fluoroquinolones and other quinolones in food-producing animals and resistance in invasive *E. coli* in humans and between resistance in *E. coli* from food-producing animals and from humans. These positive associations should be interpreted with caution, as the fraction of *E. coli* from blood-stream infections originating from domestically produced food-producing animals and meat derived thereof is not well understood. Other sources could include for example consumption of meat produced outside the reporting country, consumption of food from non-animal sources, environmental exposure, travel or direct transmission between humans.

According to the multivariate analyses for *Campylobacter* spp. and *Salmonella* spp., the consumption of fluoroquinolones in animals had a significant impact on resistance in these bacteria from food-producing animals. This association was also found in the univariate analysis, although the results for *Salmonella* spp. were generally inconclusive. The multivariate analysis further showed that the main effect on resistance to fluoroquinolones in these bacteria in humans comes from resistance in animals. As *Salmonella* spp. and *Campylobacter* spp. are for the most part zoonotic bacteria, these findings appear logical. In the univariate analysis for *Salmonella* spp., no significant associations were

however found between resistance in bacteria from broilers and from humans. This could indicate that broilers only contribute to part of the resistance to fluoroquinolones in *Salmonella* spp. in humans but it could also for example reflect the limited data available and/or the effect of clonal spread in *Salmonella* spp.

Polymyxins

Polymyxin consumption in humans is low in all EU/EEA MSs. Considering trends between 2011 and 2015 however, a significant increase in colistin consumption in the hospital sector has been recorded in several countries (ECDC, 2016c), most likely in consequence of MDR, carbapenem-resistant Gram negative infections becoming more common. In countries with high levels of multi-drug resistance, including resistance to carbapenems, only a few therapeutic options remain available, among which colistin represents a last option substance. Half of the consumption of colistin in humans occurs in the hospital sector, where it is reserved for treatments of MDR Gram negative infections. In the community, colistin is used for the treatment or prophylaxis of infections in patients with cystic fibrosis, as many of them commonly carry MDR bacteria. Although data on colistin resistance are not complete in the EARS-Net surveillance database (because not all countries are testing for colistin resistance), the observations that countries with currently high percentages of carbapenem resistance report large numbers of isolates with combined carbapenem and colistin resistance are indicative of the further loss of effective treatment options for Gram-negative bacterial infections.

Colistin resistance in both invasive *E. coli* and invasive *K. pneumoniae* from humans, although very low, was associated with consumption in humans. The association between consumption of polymyxins in humans (especially at the hospital) and resistance to polymyxins in invasive *K. pneumoniae* is not surprising, as colistin use is the only independent risk factor for acquisition of colistin-resistant, carbapenemase producing *Enterobacteriaceae* in matched, controlled studies (Brink et al., 2013; Halaby et al., 2013; EMA/AMEG, 2016). When interpreting these results, it should be taken into account that data on colistin resistance in humans from EARS-Net were available mostly from isolates which were resistant to carbapenems. Therefore, they are not fully representative of the whole bacterial population of invasive *E. coli* and *K. pneumoniae* isolates.

In 2016, the CLSI-EUCAST joint Polymyxin Breakpoints Working Group published a method for determination of colistin MIC. Susceptibility testing by other methods, such as agar dilution, disk diffusion or gradient diffusion, should be disregarded until historical data have been thoroughly reviewed or new study data generated.[5] As information on methods used for susceptibility to colistin may be incomplete in EARS-Net, the impact of use of various testing methods on the results is unknown.

In the animal sector, the consumption of polymyxins was much higher than in the human sector. Colistin consumption was significantly associated with colistin resistance in indicator *E. coli* isolates from food-producing animals, though typically low, whether considering the SIMR in food-producing animals in 2014–2015, or more specifically, the occurrence of resistance in pigs in 2015 and in poultry in 2014. As data on resistance is derived from phenotypic susceptibility testing, it was not possible to differentiate between non-transferable and transferable resistance mechanisms.

Resistance to colistin in *E. coli* and *K. pneumoniae* can occur through chromosomal mutations, acquisition of a bacterial cell capsule or through acquisition of transferable resistance genes (e.g. the colistin resistance gene *mcr-1*). Recently performed studies in Germany have shown that the contribution of *mcr-1*-mediated transferable resistance to phenotypic colistin-resistance in animals, especially in poultry, can be substantial (Irrgang et al., 2016). Recognising the increasing importance of colistin to treat MDR Gram-negative infections in humans, and in order to reduce the risk of spread of transferable resistance to colistin from animal microbiota to humans, recommendations were made by the AMEG with the goal of reducing as much as possible the consumption of colistin in veterinary medicine (EMA, 2013) and differentiated targets to substantially reduce veterinary use of colistin in the EU MSs were also set up to account for the wide variations in the use of colistin in the EU MSs (EMA/AMEG, 2016).

Plasmid-borne colistin resistance resulting from acquisition of *mcr-1* has rarely been reported for *K. pneumoniae* isolates from humans (Poirel et al., 2017), whereas corresponding data on the occurrence of *mcr-1* in indicator *E. coli* from food-producing animals were not available from most MSs. Additionally, there were only very limited data on colistin resistance in invasive *E. coli* from humans available, and, if any, they related to the subpopulation of invasive *E. coli* detected as

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[5] See http://www.eucast.org/

exhibiting resistance to carbapenems. For these reasons, the comparison, whether using the univariate or the multivariate approach, of colistin resistance in indicator commensal *E. coli* from healthy animals and either invasive *K. pneumoniae* or invasive *E. coli* from humans was not performed, because the analysis must be based on plausible biological pathways of transmission of resistance.

Macrolides

In humans, macrolides are used for the treatment of infections caused by Gram-positive bacteria (e.g. in respiratory and soft tissue infections), by some Gram-negative bacteria including *Campylobacter* spp. (gastroenteritis) and by intracellular bacteria, such as *Mycoplasma pneumoniae.* In animals, macrolides (and the related substances lincosamides) are important antimicrobials for the treatment of many common infections in cattle and pigs (Pyorala et al., 2014).

No statistically-significant association was observed between macrolide consumption in humans and resistance in isolates of *C. jejuni* and *C. coli* from humans, whereas the use of macrolides in animals was statistically-significantly associated with resistance of *Campylobacte*r spp. to macrolides in isolates from both food-producing animals and humans. These results were not unexpected, as *Campylobacter* spp. are zoonotic bacteria which commonly originate from food-producing animals and exposure of animals to antimicrobials is expected to increase the resistance level to antimicrobials in those bacteria, which could later be transferred to humans.

Campylobacter spp. are not part of the normal intestinal flora in humans and therefore, rarely subject to a selective pressure in the human host. Gastroenteritis caused by *Campylobacter* spp. is mostly self-limiting and antimicrobial therapy is normally not required. Macrolides are however one of few available therapies for serious invasive campylobacter infections.

Tetracyclines

Tetracylines are not routinely used in treatment of Enterobacteriaceae infections in humans; therefore, there are no surveillance data on tetracycline resistance in invasive *E. coli* from humans. In the monitoring of resistance in *Salmonella* spp. isolates from humans, tetracyclines are included as an epidemiological marker to separate strains and to assess any possible linkage as to a potential animal origin of the resistance. For *Campylobacter* spp., tetracyclines are an option for treatment.

Overall, there were no, or very few, significant associations between consumption of tetracyclines in humans and resistance in *Salmonella* spp. and *Campylobacter* spp. from humans. This could be explained by the low use of this class of antimicrobial in humans. Conversely, in food-producing animals, where tetracyclines are the most sold antimicrobials, a statistically-significant positive association between consumption of tetracyclines and resistance in *E. coli*, *Salmonella* spp. and *C. jejuni* from such animals was observed.

Some inconsistencies were observed in the results for *Salmonella* spp. This could be due to inherent resistance traits in different salmonella serovars where e.g. 60% of *S*. Typhimurium and 90% of monophasic *S*. Typhimurium from humans were resistant to tetracyclines in 2015, whereas the corresponding figure for *S*. Enteritidis was only 3% (EFSA/ECDC, 2017). A large proportion of *S*. Typhimurium share the ASSuT resistance pattern (i.e. resistant to ampicillin, streptomycin, sulfonamides and tetracyclines), and this ASSuT clonal group is known to circulate in different European countries (Hopkins et al., 2010; Lucarelli et al., 2010). The spread of certain salmonella clones via the food-chain, international food trade or via travel, or co-selection of multidrug-resistant (MDR) strains when the bacteria are exposed to other antimicrobials may all impact on the occurrence of resistance in *Salmonella* spp. One example is tetracycline-resistant *Salmonella* spp. isolates acquired in third countries (e.g. Africa) and imported to the EU via travellers.

The multivariate analysis indicated that there is a strong association between the consumption of tetracyclines in animals on the resistance observed in *C. jejuni* from both animals and humans. The resistance data from animals included in this analysis were from poultry and poultry consumption and handling of poultry is an important transmission route for *Campylobacter* spp. infections in humans. The consumption of tetracyclines is generally higher in pigs than in poultry and to assess the impact of the consumption of tetracyclines in pigs, more resistance data on *Campylobacter coli* would be needed.

Carbapenems

Carbapenems are considered by WHO as CIAs and by AMEG classification as category 3 indicating that this class of antimicrobials should not be used in animals because of its high importance for human medicine. Accordingly, carbapenems are not authorised for use in animals in the EU.

In humans, carbapenems are almost exclusively used in hospitals and for treatment of infections caused for MDR Gram-negative bacteria. Recent data from ECDC (2011–2015) have shown that the EU/EEA population-weighted average consumption of carbapenems showed a significant increase. The same report found that six countries had a significant increase in carbapenems use and none had a significant decrease. Although carbapenem consumption is still at a relatively low level compared to the overall consumption of antimicrobials for systemic use in the hospital sector, the increasing rates combined with emergence and spread of carbapenem-resistant bacteria (including *Acinetobacter baumannii*, Enterobacteriaceae and *Pseudomonas* spp.) is worrisome.

Among Enterobacteriaceae, carbapenem-resistant *K. pneumoniae* has shown a clear trend to clonal spread causing outbreaks in healthcare settings in several EU/EEA MSs. International high-risk bacterial clones, such as the KPC-producing *K. pneumoniae* ST258, have emerged. These clones are very efficient at colonising human hosts and highly successful at spreading in hospital settings. Therefore, the large differences in the reported percentage of carbapenem-resistant *K. pneumoniae,* especially in healthcare settings, could be due to clonal spread (patient-to-patient transmission), use of carbapenems and other antimicrobials, or both.

Carbapenem use could act as a facilitator of spread of resistant clones, at least for countries where resistance has been documented. The statistically-significant associations in this report between carbapenem consumption and carbapenem resistance strongly suggest that, in some countries, consumption of carbapenems is a major driver for carbapenem-resistant *K. pneumoniae.*

In EARS-Net, carbapenem resistance was more common in *K. pneumoniae* than in *E. coli*. In most of the countries, carbapenem resistance in *E. coli* was less than 1%. The estimated burden of carbapenem resistance is therefore higher in *K. pneumoniae* than in *E. coli.* A reason for the low occurrence of carbapenem-resistant *E. coli* could be that most of the invasive *E. coli* isolates included in this analysis represent infections acquired in the community, where carbapenems are seldom used, whereas invasive *K. pneumoniae* isolates represent infections acquired in hospitals, where use of carbapenems is much more common as well as opportunities for patient-to-patient transmission.

In food-producing animals, only a limited number of carbapenemase-producing Enterobacteriaceae (CPE) has been found. In 2013, EFSA documented carbapanemase production (VIM-1) in *E. coli* and a putative strain of *Salmonella* Infantis from pigs, poultry and their environment in Germany (EFSA BIOHAZ Panel, 2013). In 2015, two single carbapenemase-producing *E. coli* isolates were reported in by two EU MSs, one from the mandatory, specific monitoring of ESBL/AmpC/carbapenemase-producing *E. coli* in fattening pigs and the other from voluntary monitoring of *E. coli* in meat at retail. No other country reported any positive findings from food-producing animals despite the high number of *E. coli* and *Salmonella* spp. isolates tested (EFSA/ECDC, 2016). Monitoring of CPE in food-producing animals and meat derived thereof using selective culture for CPE is described in Commission Decision 2013/652/EU (Official Journal of the European Union, 2013) and is voluntary, though was performed by several MSs in 2015. It may be adopted by MSs in addition to culture on selective media containing cefotaxime which is mandatory. The mandatory culture on selective media containing cefotaxime, although primarily intended to detect ESBL and AmpC-producing *E. coli*, is also selective for most (though not all) CPE. Any rise in carbapenem resistance is therefore likely to be promptly detected.

Assessing the possible effect of co-selection

Accounting for the effect of co-selection might be expected to increase the strength of the association between AMC and AMR. The analysis utilised composite indicators of AMR and AMC from different animal species; optimally the influence of the component data at the level of each animal species should also be analysed, but this was not possible with the data currently available. Furthermore, the analysis of data at individual animal species level may have particular relevance in some cases, for example, off-label use of 3rd- and 4th-generation cephalosporins is not authorised in poultry in the EU, whereas these antimicrobials are authorised for use in pigs and cattle. Aggregating data from all species may therefore not provide a composite indicator which is optimal for the analysis.

Co-selection may occur when different AMR genes are genetically linked in a bacterium. The consumption of a particular antimicrobial therefore selects for resistance not only to that particular antimicrobial but to the other genetically-linked resistances which are also present. Thus, use of a particular antimicrobial might (through co-selection) select for resistance to a different antimicrobial. Application of mathematical procedures to take account of co-selection in investigating associations between AMC and AMR in bacteria from food-producing animals resulted in decreased strength of association for 3rd-and 4th-generation cephalosporins and fluoroquinolones, with little change in the case of tetracyclines; it consistently narrowed the confidence interval of the odds ratio and two

combinations which had not previously shown statistically-significant associations (2013, tetracyclines; 2013, 3rd-and 4th-generation cephalosporins) became significant. Taking co-selection into account provided a refinement to the analysis by providing a more precise assessment of the odds ratio and resulted in statistical significance between AMC and AMR becoming apparent in some cases. A decrease in the strength of association when taking into account co-selection occurs because the "corrected" AMC figure (taking into account the co-selective effect), invariably provides a higher "corrected" AMC value which results in a decrease in the gradient of the regression line.

For animals, a further limitation of the approach taken was that the AMC data expressed in milligrams per kg of biomass (PCU) were used for this specific analysis as a proxy for AMC data expressed in numbers of DDDvet per kg of biomass (PCU). The summing of consumption of various classes of antimicrobials expressed in DDDvet allows the different dosing schedules of those classes to be accounted for; this was not taken into account using consumption data expressed in milligrams per kg of biomass (PCU). In addition, the limited amount of available AMR data reported at isolate level on poultry, pigs and bovine animals greatly reduced the number of countries which could be included in this analysis for both 2013 and 2014–2015. In 2013, the reporting of AMR data at isolate level was not mandatory, while in 2014–2015, the monitoring of AMR in bovine animals under 1 year of age was not mandatory for all EU Member States.

Accounting for the phenomenon of co-selection needs some refinement particularly in terms of data availability (number or countries reporting suitable data) to improve the robustness of the outputs obtained. Refinement could also include collection of AMC data and analysis at the animal species level, as well as including AMR which are not currently addressed by the monitoring in animals and humans.

Comparing overall antimicrobial consumption with complete susceptibility in indicator *E. coli* from food-producing animals

The OR estimates obtained by the models were consistent in both 2013 and 2014–2015, indicating that an increase in total AMC of 10 mg per kg of estimated biomass and per year resulted in an increase by 10% of the probability of detecting indicator *E. coli* (microbiologically) resistant to at least one of the substances tested. The calculations performed were slightly different in 2013 and in 2014–2015, yet the strength of the association and statistical outputs were remarkably consistent. The consistency of the outputs and the availability of data from a large number of different countries, suggest that the association between complete susceptibility and AMC is a key area for investigation in analyses of the type performed in the second JIACRA report.

It seems likely that the ability of individual antimicrobials to influence the occurrence of complete susceptibility in indicator *E. coli* is not equal; this ability might be partly dependent, for example, on the availability and molecular arrangement of resistance genes in the gut microbiota or environment which can be acquired (through gene transfer) by indicator *E. coli*. Total AMC in food-producing animals is particularly influenced by those antimicrobials which are most frequently used in animals. In that respect, tetracyclines, sulfonamides and penicillins (including aminopenicillins) may be particularly important in both influencing the acquisition of resistance genes by indicator *E. coli* and in their proportionately high contribution to the total AMC. The contribution of individual antimicrobials to the occurrence of full susceptibility is an area for possible further study.

In food-producing animals, a statistically-significant negative association was consistently detected between total AMC and the occurrence of complete (microbiological) susceptibility to all of the antimicrobials tested. The wide range in values observed both for the total AMC in animals and in the occurrence of complete susceptibility which was observed in food-producing animals in different EU MSs, provided a range of data highly suitable for analysis by logistic regression and tended to separate different MSs, rather than split them into amorphous clusters. This association was not studied in the first JIACRA report. Although the animal species included were different in the different years analysed in this second JIACRA report, this association between complete susceptibility and AMC was consistently observed.

The EC has recently mandated, in a separate request, ECDC, EFSA and EMA to establish a list of harmonised outcome indicators suitable for monitoring and detecting reductions of relevant magnitude in the levels of AMR in bacteria from humans, food-producing animals and food derived thereof, and in AMC in humans and food-producing animals (European Commission, 2016). These indicators should assist MSs to assess, in a clear and simple way, the progress made in the implementation of their action plans against AMR. This analysis addressing complete susceptibility provides information that may contribute to the rationale for the selection of appropriate indicators.

15.   Overall conclusions

The epidemiology of AMR is complex and factors other than AMC may influence the level of AMR. Differences between the systems for collection and reporting of data on AMC and AMR in bacteria from humans and food-producing animals at the time of data collection (2013–2015), unavoidably hamper direct comparisons. The caveats described in the methodology should be kept in mind when interpreting the statistically-significant associations reported here. Nevertheless, in most cases, AMC was positively associated with AMR in both animals and humans. Many of the observed findings fit well with the current knowledge of the epidemiology of AMR and infections relating to the bacterial species studied. These results confirm the need to ensure prudent use of antimicrobials and thereby reducing unnecessary consumption of antimicrobials in food-producing animals and in humans (Official Journal of the European Union, 2015; EMA/CVMP, 2016; EMA/EFSA, 2017; ECDC, 2017b).

The multivariate analysis proved to be a useful approach to assess statistical significance and relative strength of associations between the occurrence of AMR in bacteria from humans, on one hand, and AMR in bacteria from food-producing animals and AMC in both food-producing animals and humans, on the other hand. Although only performed for three of the six antimicrobial classes analysed in this report, the multivariate analysis showed that depending on the bacteria/antimicrobial substance combinations, the relative strength of the associations between consumption and resistance within the human sector and between consumption in animals and resistance in bacteria from humans, may differ markedly.

The multivariate analyses showed that for resistance to 3rd-generation cephalosporins in invasive *E. coli* from humans, there was a significant direct effect of the consumption of 3rd- and 4th-generation cephalosporins in humans, and hospital consumption appeared to have the greatest weight. No significant effect of the consumption of 3rd-generation cephalosporin in food-producing animals or the corresponding resistance in commensal *E. coli* from food-producing animals was observed. Associations between AMC and AMR were assessed using available data on the occurrence of phenotypic resistance to 3rd- and 4th-generation cephalosporins. Investigation of these associations in specific animal sub-populations, and accounting for resistance genotypes would have helped in further refining the analysis, but could not be performed because of lack of data. The multivariate analyses showed that resistance to fluoroquinolones and other quinolones in *Salmonella* spp. and *C. jejuni* from humans was significantly associated with resistance in these bacteria in food-producing animals, which, in turn, was significantly associated with the consumption of fluoroquinolones in food-producing animals. Multivariate analyses showed that resistance to tetracyclines in *C. jejuni* from humans was significantly associated with resistance in bacteria from food-producing animals. It was significantly associated to the consumption of tetracyclines in food-producing animals.

The availability of more detailed and comprehensive data would increase the scope of the analyses that can be performed and the robustness of corresponding outputs. Data should preferably be collected so that analysis of its component parts is possible (AMC in hospital *versus* community, or AMC in pigs versus cattle versus broilers versus turkeys). Availability of AMC by species and reporting of these data by DDDvet will help to address this. Additional information, including AMC by animal species and collection of AMR data from all countries, from relevant food-producing animal species, at a detailed level, including production type, is required.

The bacterial species included in this report encompass some in which spread of drug-resistant clones are considered to be highly important. This may impact on the assessment of the relationship between AMC and AMR and could explain why significant statistical associations between AMC and AMR were sometimes found and sometimes not, e.g. in *K. pneumoniae* and in some cases in *Salmonella* spp. and certain salmonella serovars. A better understanding of the relative importance of spread of resistant clones and/or influence of AMC could be gained if data on genotypic resistance and molecular typing were available for analysis. Further analysis of certain bacteria and AMR therein at the molecular level would also enhance the effectiveness of analyses between isolates from the human and food-producing animal sectors. Demonstration of identical genes encoding for AMR in bacteria in the different sectors would help to ensure that associations detected are genuine and increase the validity of the observations.

Data on AMR from commensal *E. coli* from healthy humans would most likely be a good indicator of the relative exposure to AMR bacteria through food consumption, transfer of *E. coli* between humans and other animals as well as the direct effect of AMC in the community on bacteria in humans. The ability to compare commensal *E. coli* in humans and animals might be particularly useful in a multivariate analysis. In general, multivariate analysis appears to have great potential and should be further explored. Other factors that could be considered in the analysis are resistance to other

antimicrobials (co-resistance), travel by humans, transfer of patients between hospitals, import and trade of food, food of non-animal origin, trade of live animals both between and within countries and exposure of animals or humans via the environment. The findings in ecological analyses such as those presented in this report should be considered as hypotheses for subsequent testing by focused research that could provide better explanations for some of the observed associations.

Complete susceptibility to a standardised panel of antimicrobial classes showed statistically-significant negative association with total AMC in food-producing animals; both parameters (complete susceptibility and total AMC) showing wide variation between MSs. This suggests that measures aiming at encouraging prudent use should concern all antimicrobial classes consumed so as to take into account the potential impact of the phenomenon of co-selection of AMR. These parameters and this specific analysis appear to have great potential and should be further explored.

Improvements of existing systems were highlighted in the first JIACRA report and several of these recommendations currently remain valid. They include the refinement of existing surveillance systems by providing more detailed information on AMC by age and gender in humans and by species and production types in animals and by providing enhanced data on hospital AMC in more countries. The greater availability of isolate-based data would enable analysis of the effects of co-selection in more sectors. For the zoonotic pathogenic bacteria, a larger number of tested isolates – still accounting for the prevalence - should be provided by each country and more countries should report data from both the animal and human sectors. Any improvement of data collection should be coordinated between the different surveillance networks, with the overarching aim of integrated analysis of the data. Monitoring of AMR should also include animal pathogens, commensal flora from both healthy humans and humans with infection, and information about the origin of the animals, particularly where importation from other sources may influence the occurrence of AMR.

Finally, it is important that the findings of this report are used to promote responsible use of antimicrobials in both humans and animals, by all stakeholders. Decreasing unnecessary use of antimicrobials will result in reductions of AMC, which in turn is expected to have a beneficial impact on the occurrence of AMR.

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Abbreviations

95% PL CI	profile likelihood confidence interval, 95%
AMC	antimicrobial consumption
AMEG	Antimicrobial Advice ad hoc Expert Group
AMR	antimicrobial resistance
AST	antimicrobial susceptibility testing

ATC	anatomical therapeutic chemical
ATCvet	anatomical therapeutic chemical animals
BI	business intelligence
BIOHAZ	EFSA Panel on Biological Hazards
BSI	bloodstream infections
CHMP	Committee for Medicinal Products for Human Use
CI	confidence intervals
CIA	critically important antimicrobial
CLSI	Clinical and Laboratory Standards Institute
CPE	carbapenemase-producing Enterobacteriaceae
CRE	Carbapenem-resistant Enterobacteriaceae
CSS	caecal samples from healthy animals at slaughter
CVMP	Committee for Medicinal Products for Veterinary Use
DDD	defined daily dose
DDDvet	defined daily doses for animals
DT	definitive phage type
EARS-Net	European Antimicrobial Resistance Surveillance Network
ECDC	European Centre for Disease Prevention and Control
ECOFF	epidemiological cut-off value
EEA	European Economic Area
EFSA	European Food Safety Authority
EMA	European Medicines Agency
EQA	External Quality Assessment
ESAC-Net	European Surveillance of Antimicrobial Consumption Network
ESBL	extended-spectrum beta-lactamase
ESVAC	European Surveillance of Veterinary Antimicrobial Consumption
EU	European Union
EUCAST	European Committee on Antimicrobial Susceptibility Testing
FAO	Food and Agriculture Organization
FAOSTAT	FAO Statistical Databases
FWD-Net	Food- and Waterborne Diseases and Zoonoses Network
HIA	highly important antimicrobial
JIACRA	Joint Interagency Antimicrobial Consumption and Resistance Analysis
MAH	marketing authorisation holder
MDR	multidrug-resistant
MIC	inhibitory concentration
MRSA	methicillin-resistant *Staphylococcus aureus*
MS	Member State
MU	million units
MVA	multivariate analysis
NCP	national control plans
NPHRL	National Public Health Reference Laboratories
OR	odds ratio
PCU	population correction unit
PL CI	profile-likelihood confidence interval
PLS-PM	Partial Least Squares Path Modeling
SIMR	summary indicator of microbiological resistance
SPC	Summary of the Product Characteristics
STEC	Shiga-toxin-producing *Escherichia coli* (synonymous with VTEC)
TESSy	The European Surveillance System
UD	unit dose
VMP	veterinary medicinal product
VTEC	verocytotoxin-producing *Escherichia coli* (synonymous with STEC)
WHO	World Health Organization
WHO CC	World Health Organization Collaborating Centre

List of countries

Austria	AT
Belgium	BE
Bulgaria	BG
Croatia	HR
Cyprus	CY
Czech Republic	CZ
Denmark	DK
Estonia	EE
Finland	FI
France	FR
Germany	DE
Greece	EL
Hungary	HU
Iceland	IS
Ireland	IE
Italy	IT
Latvia	LV
Lithuania	LT
Luxembourg	LU
Malta	MT
Netherlands	NL
Norway	NO
Poland	PL
Portugal	PT
Romania	RO
Slovakia	SK
Slovenia	SI
Spain	ES
Sweden	SE
Switzerland	CH
United Kingdom	UK

Appendix A – Information available in JIACRA I

A.1.   Conclusions from JIACRA I

To analyse the relationship between consumption of antimicrobials and resistance in bacteria from food-producing animals, a summary indicator of resistance in the main three food-producing animal species was calculated on the basis of the weighted mean by PCU of the proportions of resistant bacteria in each of those animal species. Overall, a positive association was observed between AMC in food-producing animals and occurrence of resistance in bacteria from such animals for most of the combinations investigated. The strongest associations between consumption and resistance in food-producing animals were found for the antimicrobials studied in relation to indicator *Escherichia coli*. Positive associations were also noted for *Salmonella* spp. and *Campylobacter* spp.

A positive association was observed between the total consumption of 3rd- and 4th-generation cephalosporins in humans and the occurrence of resistance to 3rd-generation cephalosporins. A positive association was also observed between the total consumption of fluoroquinolones in humans and the occurrence of fluoroquinolone resistance in *E. coli* from humans. No association was found between the consumption of fluoroquinolones in humans and the occurrence of fluoroquinolone resistance in *Salmonella* spp. or in *S.* Enteritidis and *S.* Typhimurium isolates from cases of human infection.

For both cephalosporins and fluoroquinolones, positive associations were found between occurrence of resistance in indicator *E. coli* originating from food-producing animals and the occurrence of resistance in *E. coli* from humans.

No associations were observed between the consumption of 3rd- and 4th-generation cephalosporins in food-producing animals and the occurrence of resistance to this sub-class in selected bacteria from humans. No associations were observed between the consumption of fluoroquinolones in food-producing animals and the occurrence of resistance in *Salmonella* spp. and *Campylobacter* spp. from cases of human infection.

Positive associations were noted for consumption of macrolides in food-producing animals and the occurrence of resistance in *Campylobacter* spp. from cases of human infection, and for consumption of tetracyclines and the occurrence of resistance in *Salmonella* spp. and *Campylobacter* spp.

In the reported analyses, associations between the consumption of selected combinations of antimicrobials and the occurrence of resistance in bacteria were observed for most of the combinations addressed in humans and animals. JIACRA I noted that the epidemiology of resistance is complex, and several factors aside from the amount of AMC influenced the level of resistance.

Differences between the systems for collection and reporting of data on AMC and resistance in bacteria from humans and food-producing animals, at the time of data collection (2011–2012) hampered direct comparisons. Owing to the characteristics of these data, the interpretation criteria and differences in units of measurement, JIACRA I noted that the results which indicate associations of potential concern should be interpreted with caution.

A.2.   Matters concerning the development and zoonotic spread of AMR considered in JIACRA I

A.2.1.   Clonal spread of bacteria

Clonal spread of bacteria conferring resistance to antimicrobials occurs in both food production animals and in humans, and in both pathogens and non-pathogens, and is not necessarily directly linked to the use of antimicrobials in a particular host or country. The implications of clonal spread in assessing the prevalence of resistance are considerable, and pose difficulties for comparative studies of resistance in human and animal populations in relation to AMC. For example, the clonal spread of a MDR strain of *Salmonella* spp. through a food animal population can be quite different from that of a strain of, e.g. MRSA in the community or in a hospital environment.

Examples of clonal spread

The appearance and subsequent epizootic spread, in the late 1980s/1990s, of a clone of *S.* Typhimurium definitive phage type (DT) 104, exhibiting chromosomally mediated resistance to five unrelated antimicrobials provides an example of clonal spread.

A further example is provided by the ongoing multicountry spread of a MDR 'clonal complex' of a monophasic variant of *S.* Typhimurium (*S.* 4,[5],12:i:-) in pig populations, exhibiting chromosomally mediated resistance to four unrelated antimicrobials which has spread extensively in pigs in several European countries, and has also caused many human infections.

MDR *Salmonella* spp. are prevalent in pigs in most countries, and are likely to have resulted from the regular use of in-feed antimicrobial treatments that are used to prevent disease, in some cases under sub-optimal husbandry conditions. The international dissemination of *S.* 4,[5],12:i:- in pig populations is likely be related to the selective advantage offered by MDR profiles associated with stable genetic elements, also carrying virulence features, within bacterial lineages that are well adapted to the porcine host and are prevalent in human infections as a result of contaminated pig meat.

The clonal spread of a strain of *S.* Kentucky ST 198 exhibiting high level resistance to ciprofloxacin and associated with poultry has recently reported to be spreading epidemically, with infections reported in several EU countries and the USA.

Clonal spread of MDR *E. coli* pathogenic to humans is an increasingly important issue, particularly in relation to the spread of ESBL-producing strains in the community and in hospitals. The potential contribution of food-producing animals or foods to public health risks by ESBL-producing bacteria is related to specific plasmid-mediated ESBL genes encoded by a number of organisms and to the subsequent horizontal dissemination of such genes through bacteria in human and animal populations. Although there are a large number of genes which encode ESBL enzymes not all are equally prevalent among human and animal bacteria. The predominant ESBL families encountered are CTX-M, TEM and SHV and the bacterial species most commonly identified with these genes in humans is *E. coli*, particularly clonal lineages: B2-*E. coli* O25:H4-ST131, D-*E. coli* O25a-ST648 and D-*E. coli*-ST69, -ST393.

A.2.2. Increasing levels of resistance to 3rd- and 4th-generation cephalosporins through production of extended spectrum β-lactamase (ESBL) enzymes in bacteria from community patients and livestock

Since 2000, a variety of plasmid-mediated β-lactamases, have emerged in Gram-negative bacteria, resulting in reduced susceptibility to broad spectrum β-lactams. These β-lactamases included both ESBLs and AmpC β-lactamases (AmpC). The multiresistant nature of bacteria that produce ESBLs and AmpCs can affect the selection and timely administration of appropriate antimicrobials for community-acquired and healthcare-associated infections, since many first-line antimicrobials are no longer active against them. Although person-to-person spread is recognised as the main method of spread of ESBL/AmpC-β-lactamase-containing *E. coli* both in hospitals and the community, the primary reservoirs of such organisms are contentious. ESBL-producing *E. coli* have been isolated from food-producing animals in many European countries, particularly poultry and cattle, and farm animals are now recognised as important carriers of ESBL/AmpC-producing *E. coli* and *Salmonella* spp. Similarly there have been an increasing number of reports of ESBL-producing *E. coli* being isolated from foods of animal origin.

Appendix B – Methods

B.1. Comparison of how antimicrobials are used in food-producing animals and in humans

Table B.1 summarises some of the differences in how antimicrobials are used in humans and in food-producing animals.

Table B.1: The use of antimicrobials in humans and in food-producing animals

	Humans	Food-producing animals and products thereof	Comments
Patient characteristics			
Species	One	Many	–
Number of individuals	5.1×10^8 (EU-28 countries, year 2016[a])	19×10^8 (EU-28 countries, year 2014[b])	Ratio humans/animals: about 1–4
Biomass (kg)	3.2×10^{10} kg (number of individuals in the EU-28 countries in 2016 multiplied by 62.5 kg/person)	Meat: 4.5×10^{10} kg Eggs: 0.7×10^{10} kg Milk: 16×10^{10} kg Honey: 0.02×10^{10} kg (EU-28 countries, 2014[c]) Fish: 0.26×10^{10} kg (EU-28 countries, 2014[d]) Total: 21.5×10^{10} kg	Ratio human biomass/animal meat[e]: 42%/58 %
Individual weight	Variable	Very variable	Animals can be treated with doses of 50 g (1-day-old chicks) up to 1,000 kg
Lifespan	Long	Short in most cases	Food animals are consumed by humans as food
Conditions for treatment			
Individual treatment	Yes	Yes	Companion animals, horses, dairy cows, adult cattle, adult pigs
Group treatment	Exceptional	Yes	Group treatment on farms
Route of administration	Oral (e.g. tablets, syrup), injectables and others	Oral (in feed or drinking water), injectables and others	Medicines for animals are focussed into efficient administration for group treatment

Adapted from 'Antibiotiques en médecine vétérinaire: caractéristiques et évolution de l'exposition des animaux d'après les données du système national de surveillance' (G. Moulin, Anses-ANMV, 2013; http://social-sante.gouv.fr/IMG/pdf/Exposition_des_animaux_aux_antibiotiques_donnees_du_systeme_national_de_surveillance-2.pdf).
(a): Eurostat: http://epp.eurostat.ec.europa.eu/tgm/table.do?tab=table&init=1&plugin=1&language=en&pcode=tps00001
(b): FAOSTAT live animals. Heads of cattle, buffaloes, sheep, goats, pigs, horses, assess, mules, chickens, ducks, geese, guinea fowls, turkeys, rabbits, hares, pigeons and other birds. No fish or bees included. http://faostat3.fao.org/download/Q/QA/E
(c): FAOSTAT livestock primary. Kilograms of indigenous cattle, buffalo, sheep, goat, pigs, horse, mule, ass, chicken and other poultry. Fish production not included. http://faostat3.fao.org/download/Q/QL/E
(d): FAOSTAT aquaculture. Fish, crustaceans, molluscs and others.
(e): The ratio excludes eggs, milk, honey and fish.

B.2. Method to calculate human consumption indicators for antimicrobial classes (expressed in DDD and per weight of antimicrobial substances)

B.2.1. Reporting of human antimicrobial consumption data to ESAC-Net

Member States have two options reporting national representative consumption data to ECDC.

- The preferred standard option, i.e. reporting of national AMC data at the medicinal product level and expressed as a number of packages sold. For this option, a valid national register of available antimicrobials is required (national registry data).

- A 'light' version, i.e. when national registry data are not available, reporting of aggregated numbers of DDD from national AMC data at the ATC substance level.

Two-thirds of the Member States reported the standard version. After reporting of the data by nominated national contact points and passing in-built validation rules of The European Surveillance System (TESSy) for the ESAC-Net metadata each country checks own data for consistency by comparing data displayed in TESSy online reports with data from national sources. The final data validation is performed by TESSy data managers and ECDC experts.

The reporting of AMC is done at the substance level (ATC codes, 5th ATC group level) including information on the route of administration (e.g. oral or parenteral). The allocation of defined daily doses (DDD) by the WHO Collaborating Centre for Drug Statistics Methodology (WHO CC) for some ATC codes depending on specific salt ingredients and the route of administration as per inhalation the galenic form (solution, powder) is an indispensable information.

The current report comprises ESAC-Net AMC data of the ATC group J01, antimicrobials for systemic use, reported for the years 2013–2015. As the annual surveillance data for a specific year are reported the year after, the respective 2014, 2015 and 2016 versions of the ATC/DDD index from the WHO CC were applied accordingly. The latest ATC/DDD index is available at http://www.whocc.no/atc_ddd_index and contains all valid ATC codes and corresponding DDD. Changes between different ATC/DDD indexes (e.g. between a current year and previous years) can also be found there.

The numbers of DDD reported for a specific antimicrobial (substance level, 5th ATC group level) are derived from TESSy calculation for those countries reporting the standard version based on the numbers of items (e.g. tables), their strength and strength unit and the respective DDD/ATC allocations by the WHO CC. Countries using the Light version of ESAC-Net for reporting are already reporting aggregated DDD numbers per ATC code and the route of administration.

There is one exception in ESAC-Net applied for all reported surveillance data up to 2015 surveillance data, where the ATC/DDD index is not followed. For so called 'combined products' for which the strength is expressed in unit doses (UD) and for which the WHO CC defines different DDD allocations for the same ATC codes at the 5th level based on the composition of the combined product.

For five 'combined products' and their respective routes of administration of the ATC group J01 and included in this report, ESAC-Net is applying fixed DDD allocation and the units are expressed in grams. These are listed in Table B.2:

Table B.2:   ESAC-Net specific DDD allocations for combined products used to recalculate 2013/2014 human AMC into weights

ATC Code	Route of administration	DDD
J01EE01	Oral	1.92 g
J01EE01	Parenteral	1.92 g
J01EE02	Oral	1 g
J01EE03	Oral	1.92 g
J01EE03	Parenteral	1.92 g
J01EE06	Oral	0.7 g
J01EE07	Oral	0.8 g

More details on reporting AMC data from Member States to ESAC-Net and about the ESAC-Net metadata can be found in Section 3.1 and in the annually updated ESAC-Net reporting protocol at the ESAC-Net webpages.[6]

B.2.2.   ESAC-Net consumption indicator expressed in DDD per 1,000 inhabitants and per day

ESAC-Net 2013–2015 AMC data expressed in DDD per 1,000 inhabitants and per day were utilised throughout the report for the logistic correlation analyses (Section 11) and for the multivariate analysis.

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[6] http://ecdc.europa.eu/en/healthtopics/antimicrobial-resistance-and-consumption/antimicrobial-consumption/esac-net/pages/esac-net.aspx

The denominator data needed to calculate DDD per 1,000 inhabitants and per day are the same for data reported for the community and for the hospital sector. It is in both cases the total population under surveillance. It shall be noted that by default TESSy is using Eurostat population data, except the Member States are using their own national population data.

AMC results expressed in DDD per 1,000 inhabitants and per day and down to the 4th ATC group level are publicly available at the ESAC-Net interactive database at the ESAC-Net webpages.[6]

B.2.3. ESAC-conversion of DDD to kg active substance

Based on a frozen TESSy dataset (status from January 2017; data reported for 2013–2015) of all ESAC-Net AMC data reported by the Member States for the community and the hospital sector, the numbers of consumed DDD at the antimicrobial substance level (5th ATC group level) were recalculated according to the ATC/DDD index of the WHO CC as described in Appendix B.2.1.

Each line of the of the dataset contained information (variables) on the ATC codes (5th ATC group level), the routes of administration including the inhalation form in case of the route of inhalation, the type of salt if applicable, and the unit of measurement (e.g. grams, mg, international units) and the numbers of units defining one DDD.

The dataset allowed recalculation of the weight of the antimicrobial at the substance level based on the numbers of DDD. The weight sums of the ATC codes were grouped into the respective antimicrobial classes according to the Anatomical Therapeutic Chemical (ATC) classification. The weight was expressed in tonnes (e.g. Tables 5 and 51 of the report) or in mg/kg human biomass using the population under surveillance and the standard human body weight (Annex B.3).

For the weight calculations, only DDD allocations for ATC codes were taken into consideration, as part of the DDD/ATC index of the WHO CC.

The ESAC-Net metadata allow Member States to report own defined national doses for those very few ATC codes and routes of administrations where no DDD allocations are defined by the WHO CC. These data could not be recalculated into weights. These amounts in weight are, however, insignificant. Based on calculated estimates the total amount in weight of all antimicrobial classes were on average less than 50 kg per year and country.

Because DDD allocation for colistin (ATC code J01XB01) is defined in million units (MU) and not in grams or mg, a conversion factor was applied to calculate the weight of consumption expressed in DDD. In humans, colistin is nearly exclusively used as colistin methane sulfonate with a concentration of 12,700 IU/mg (EMA/ESVAC, 2016b; Theuretzbacher, 2014). Therefore, a conversion factor of 1 MU = 78.74 mg was applied.

B.3. Calculation of standard human body weight

B.3.1. Introduction

The authors reached the consensus that, with data currently available, mg per kg of body weight is an acceptable unit of measurement to compare AMC in the food-producing animal and human sectors. For food-producing animals, the PCU was used for the calculations. For the human sector, a standardised body weight taking into account the distribution of the population (children, adult, the elderly, men and women) was used. Data on international human body weights are scarce. For instance, in relation to AMC, the definition of the DDD mentions that it is based an adult of 70 kg. In addition, although there are many publications on body mass index and obesity, they do not provide data on body weight. For this reason, the authors made the decision to estimate a standard human body weight from published EU data.

B.3.2. Existing data

In its scientific opinion entitled 'Guidance on selected default values to be used by the EFSA Scientific Committee, Scientific Panels and Units in the absence of actual measured data' (EFSA, 2012), EFSA proposed standard body weights for adults and children. These standard body weights were defined based on a review of EFSA publications and surveys. For adults, the standard body weight was defined as 70 kg. For children, different body weights, depending on age, were proposed (Table B.3).

Eurostat publishes data on the EU population by age and gender for all MSs and for the whole EU. These data are available in the Eurostat table entitled 'demo_pjan'.

Table B.3:   Proposed standard body weights for children by EFSA

Age (years)	Mean (kg)
Infants (0–3 months)	4.8
Infants (3–6 months)	6.7
Infants (6–12 months)	8.8
Toddlers (1–3 years)	11.9
Other children (3–10 years)	23.1
Adolescents (10–14 years)	43.4
Adolescents (14–18 years)	61.3

B.3.3.   Methodology

To compare AMC between humans and food-producing animals, the following methods, based on data provided by EFSA and Eurostat were applied to define a standard human body weight:

1) An average body weight for children below 1 year of age was calculated as Eurostat provides population data only by year.
2) An average body weight for children aged 1–18 years (including toddlers, other children and adolescents as defined in Table B.3 was calculated,
3) A standard body weight for humans was calculated using the proposed adult body weight and the calculated average child body weight.

The Eurostat population for the EU-27 in 2012 was used as reference data for the population.

Average body weight for children below 1 year of age

The average weight for children below 1 year of age was calculated by taking a weighted mean of the proposed body weights of the three categories and using the number of months of each age category as weight.

Average body weight for children

The average body weight for children was obtained by calculating a weighted mean of the calculated average body weight for children below 1 year of age and the proposed body weights for the categories of children above 1 year of age and using the number of children in each category extracted from Eurostat as weight for the mean.

To estimate a standard body weight for children from 0 to 18 years of age, the weighted mean of the EFSA proposed body weight by class of children from 1 to 18 years of age and of the aforementioned calculated body weight for children below one year was computed. The Eurostat population figures were used to weight the different classes of children. The standard body weight for children was estimated as 34.6 kg.

Standard human body weight

The standard human body weight was calculated by applying the weighted mean of the average child body weight (34.6 kg) to the population below 20 years of age and the proposed 70 kg for the population older or equal to 20 years of age and using the corresponding population figures extracted from Eurostat as weight for the mean.

Based on this methodology, the calculated standard human body weight was 62.5 kg.

B.4. Technically derived estimates of the sales of veterinary antimicrobials for pigs and poultry

B.4.1. Sales datasets

B.4.1.1. Antimicrobial VMPs included

Sales data on each antimicrobial VMPs presentation sold belonging to ATCvet groups QA07AA and QJ01[7] were derived from the ESVAC database by country and year. The selected ATCvet groups cover antimicrobial VMP for oral administration and injectables. Tablets were not included in the datasets as these are assumed to be used almost solely for companion animals (Table B.4).

Table B.4: 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 systemic use	QJ01

B.4.1.2. Identification of authorised target species

For each of the antimicrobial VMP presentations in the included datasets, information regarding the authorised target species was identified mainly by use of the national Summary of Product Characteristics (SPC). In cases where an SPC could not be located or if the information was unclear, the relevant Member State was asked to provide this information. Note that for some VMPs the SPC data indicated poultry as target species and in order to have harmonised data across MSs, sales were estimated for poultry. The AMR data covers bovine below 1 year but since cattle in general is typically given as target species in SPC's sales for bovines < 1 year could not be estimated by the approach used.

Sales data of VMPs solely indicated for use in animals other than pigs and poultry were excluded for the further analysis (e.g. those containing pradofloxacin and cefovecin).

B.4.2. Estimation of antimicrobial sales for pigs and poultry

B.4.2.1. Weighting of the sales

For each country and year, the sales of each included antimicrobial VMP presentation were distributed to species by weighting according to the biomass (population correction unit(PCU)) ratio of pigs and/or poultry and other species. The biomass ratio was defined as the fraction of the biomass (PCU) of pigs and poultry, respectively, of the total biomass (PCU) animals potentially at risk of receiving antimicrobial treatment.

The animal species (and categories) included in the calculation of PCU used to report the ESVAC sales data[8] are cattle, pigs, poultry, sheep, goat, fish, rabbits and horses. The data sources used, animal categories included and the methodology for the calculation of PCU are described in Appendix 2 of the Agency's report 'Trends in the sales of veterinary antimicrobial agents in nine European countries: 2005–2009' (EMA/ESVAC, 2011).

An example of calculation of the biomass ratio is given in Figure B.1.

$$\text{Equation 1. } Pig\ biomass\ ratio\ = \frac{Biomass\ (PCU)\ pigs\ (kg)}{\sum Biomass\ (PCU)\ all\ species(kg)}$$

Figure B.1: Example of calculation of the animal biomass ratio of each antimicrobial VMP presentation authorised for pigs

B.4.2.2. Calculation of weighted sales

The sales (weight of active substance) for pigs and poultry were calculated by multiplying the total sales of each VMP presentation by the animal biomass ratio of each of these species (Figure B.2); the

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[7] www.whocc.no/atcvet
[8] http://www.ema.europa.eu/ema/index.jsp?curl=pages/regulation/document_listing/document_listing_000302.jsp

outputs of these calculations were subsequently aggregated to sales by antimicrobial class or subclass and form for each of the three species.

$$\text{Equation 2. } Weighted\ sales\ pigs\ = Biomass\ ratio\ pigs\ \times Total\ sales\ VMP\ presentation\ X\ (mg)$$

$$\text{Equation 3. } Weighted\ sales\ poultry\ = Biomass\ ratio\ poultry\ \times Total\ sales\ VMP\ presentation\ X\ (mg)$$

Figure B.2: Calculation of weighted sales for an antimicrobial VMP presentation (X) authorised both for pigs and poultry

Example:

- Annual sales of antimicrobial VMP X authorised for pigs and poultry in country Y = 100 mg of active ingredient Z
- Biomass all target species = Biomass pigs $(1 \times 10^8$ kg) + biomass poultry $(0.5 \times 10^8$ kg) = 1.5×10^8 kg
- Weighted sales by target species:

— Pigs = $(1 \times 10^8/1.5 \times 10^8) \times 100 = 66.7$ mg
— Poultry = $(0.5 \times 10^8/1.5 \times 10^8) \times 100 = 33.3$ mg

B.4.2.3. Estimating a proxy for antimicrobial exposure in pigs and poultry

The estimated sales (weight of active substance) for each antimicrobial substance and form for each of the three species were used to calculate the numbers of defined daily dose animals (DDDvet) sold. The DDDvet[9] values, established by EMA, provide standardised fixed units of measurement for the reporting of data on consumption and takes into account differences in daily dosing between the various species, antimicrobial substances and forms (in this case oral and injectable forms) between the various species (here pigs and poultry) and has been assigned by kg of animals.[10]

$$\text{Equation 4. } \frac{\text{Numbers DDDvet (kg)}}{\text{Biomass species (kg)}}$$

Figure B.3: Indicator expressing exposure to an antimicrobial substance

The indicator used to express exposure of pigs and poultry, respectively, to the selected antimicrobials is number of DDDvet per kilogram of animal biomass (species) per year (Figure B.3).

Formula and examples for calculation of exposure are shown in Figure B.4.

$$\text{Equation 5. } \frac{Sum\ of\ sales\ of\ oral\ antimicrobial\ X\ pigs\ (\text{mg})/DDDvet\ oral\ pigs\ (\text{mg/kg})}{Biomass\ (PCU)\ pigs\ (\text{kg})}$$

$$\text{Equation 6. } \frac{Sum\ of\ sales\ of\ injectable\ antimicrobial\ X\ pigs\ (\text{mg})/DDDvet\ injectable\ pigs\ (\text{mg/kg})}{Biomass\ (PCU)\ pigs\ (\text{kg})}$$

Figure B.4: Example on calculation of exposure of pigs to antimicrobial X for administration orally and by injection, respectively

Example:

Annual sales of antimicrobial injectable X for pigs in country Y = 2×10^9 mg

- DDDvet of antimicrobial injectable X = 10 mg/kg
- Biomass of pigs in country Y = 1×10^8 kg
- $(2 \times 10^9/10)/1 \times 10^8 = 2$ DDDvet per kg animal

The outputs of the calculations were subsequently aggregated by antimicrobial class or subclass and species and by year and country.

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[9] DDDvet list can be found here: http://www.ema.europa.eu/ema/index.jsp?curl=pages/regulation/general/general_content_001493.jsp&mid=WC0b01ac0580a2fcf5
[10] See http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2015/06/WC500188890.pdf

It should be noted that the methods described only provide a crude estimate of antimicrobial sales per species and numbers of DDDvet per kilogram of animal biomass per year cannot be assumed to represent actual animal exposure to antimicrobials in the countries considered in the report. This indicator is a proxy for animal exposure to antimicrobials used in this second for the analysis of the association between AMC and the occurrence of resistance.

B.4.3. Examples of the calculations used to distribute antimicrobial sales by target animal species and estimating antimicrobial exposure in pigs and poultry

All the examples provided below are only for illustrative purposes and do not reflect the sales of any product/antimicrobial substance in the participating countries.

Example 1: Weighting of sales

Tables B.5 and B.6 provide examples on weighting of sales for an injectable and an oral VMP.

Table B.5: Weighting of sales of an injectable VMP

Target species	Pigs	Poultry	Sheep, goats
Biomass (1,000 tonnes)	380	40	80
Total biomass (1,000 tonnes)	500		
Animal biomass ratio	0.76	0.08	0.16
Sales (kg of active ingredient)	25		
Weighted sales by animal species (kg of active ingredient)	19	2	4

Table B.6: Weighting of sales of an oral VMP

Target species	Pigs	Poultry	Sheep, goats
Biomass (1,000 tonnes)	380	40	80
Total biomass (1,000 tonnes)	500		
Biomass ratio	0.76	0.08	0.16
Sales (kg of active ingredient)		10	
Weighted sales by animal species (kg of active ingredient)	7.6	0.8	1.6

Example 2: Calculation of #DDDvet per kilogram animal biomass per year

An example on calculation of outputs for pigs and poultry is given in Table B.7. The weight of active ingredient used in the calculations corresponds to the sum of the sales by animal species of all VMPs (orals and/or injectables) containing the antimicrobial substance A.

Table B.7: Example calculation of weighted sales, of numbers (#) of DDDvet and of exposure (in DDDvet per kg of estimated biomass and per year) of pigs and poultry for antimicrobial substance A and the variables used

Antimicrobial substance A			
Animal species in which it is used	Pigs	Poultry	Sheep, goats
Weighted sales by animal species (kg of active ingredient)	150	20	45
DDDvet (mg/kg)	2.5	10.0	Excluded from calculations
# DDDvet active ingredient	60,000,000	2,000,000	
Biomass of each animal species (1,000 tonnes)	380	40	
Exposure by animal species (DDDvet per kg of estimated biomass and per year)	0.16	0.05	

B.5. Principle of the calculation method of the 'corrected' consumption of an antimicrobial substance

For the purpose of modelling, the relationship between consumption and AMR to a given **Substance A**, various fractions of the consumption of those antimicrobials: **Substance B**, **Substance C**, etc associated to the multidrug resistance traits observed in the bacterial population under study (in this case, indicator *E. coli* prevalent in the animal populations addressed) should be taken into account.

B.5.1. Definition of coupling fraction

The coupling fraction cf_{BA} for **Substance B** to explain resistance to **Substance A** is defined as the percentage of *E. coli* isolates resistant to A that were also detected as resistant to **Substance B**. The percentage of isolates (resistant to **Substance A** that were also resistant to **Substance B**) needed to calculate the coupling fraction cf_{BA} were derived from the isolate-based data reported by the reporting countries on indicator *E. coli* from cattle, broilers and pigs for the year 2013 and from broilers, turkeys, pigs and veal calves for the years 2014–2015. In addition, the fractions accounted for each animal population addressed by weighting them according to their PCU.

B.5.2. Calculation of the corrected consumption

The corrected consumption of antimicrobial **Substance A**, **corrConsumption$_A$**, was then calculated using the following formula, where:

- **Consumption$_A$** is the quantity of antimicrobial **Substance A** sold (expressed in milligrams per kilogram of estimated biomass),
- **Consumption$_B$** is the consumption of antimicrobial **Substance B** sold, and
- cf_{BA} is the coupling fraction for **Substance B** to explain resistance to Substance A, etc.:
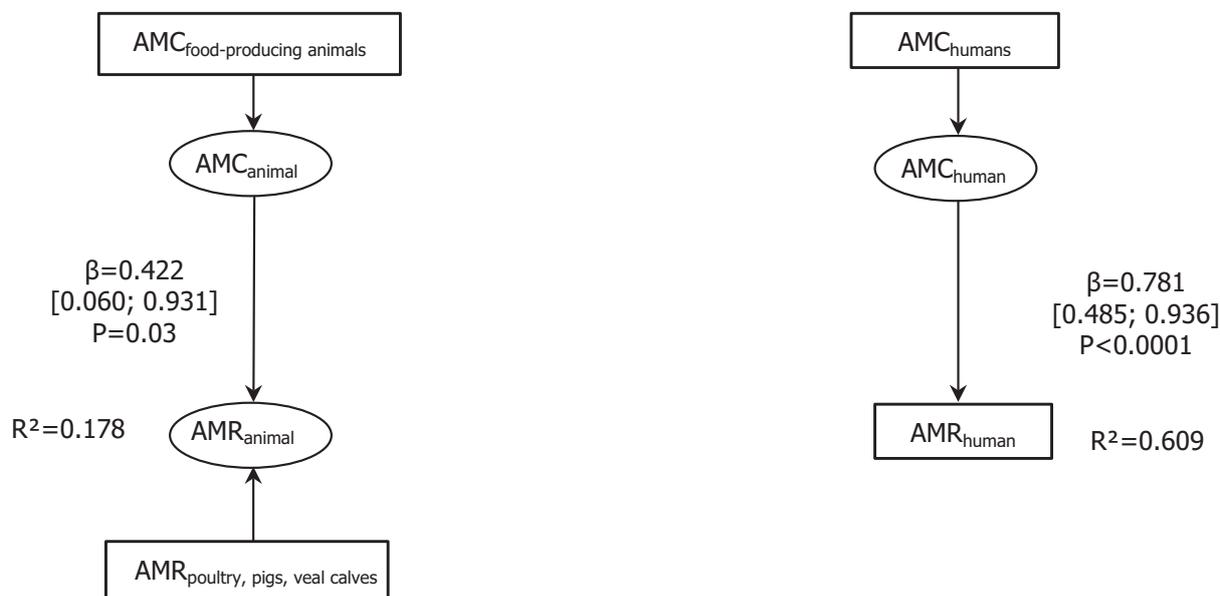
26 countries: AT, BE, BG, CY, CZ, DE, DK, EE, ES, FI, FR, HR, HU, IE, IT, LT, LV, NL, NO, PL, PT, RO, SE, SI, SK, UK.

Figure B.5: Diagram of the PLS-PM of resistance to 3rd- and 4th-generation cephalosporins in human invasive *E. coli* (2014 and 2015) considering resistance to 3rd- and 4th-generation cephalosporins in indicator *E. coli* from food-producing animals (pigs and calves < 1 year in 2015 and poultry in 2014), consumption of 3rd- and 4th-generation cephalosporins in humans (2014–2015 average, expressed in mg/kg of biomass), in animals (2014–2015 average, food-producing animals, expressed in mg/kg PCU)

B.6. Multivariate models performed on summary indicators

Deviating from the usual multiblock nature of the data, an attempt to fit models to summary indicator parameters is presented below. These models only concern *E. coli*, as *Salmonella* spp. data were available in six countries only, which did not allow fitting a multivariate model.

B.6.1. Cephalosporins

As observed in PLS PM models performed on multiblock manifest variables, a strong significant relationship was identified were between 3rd- and 4th-generation cephalosporins consumption in humans and resistance in invasive *E. coli* from humans (both path coefficients and R^2 were of the same magnitude). In the present model, a significant relationship was also observed between 3rd- and 4th-generation cephalosporins consumption in food-producing animals and resistance in food-producing animals. The fraction of the variance of resistance explained was nevertheless reduced ($< 20\%$). Furthermore, sensitivity analysis showed that, when not considering two countries, this relationship was no more significant (Figure B.5).

It is of note that, in such a model, path coefficients can directly be related to correlation coefficients between variables.

B.6.2. Fluoroquinolones

The indirect effect of animal fluoroquinolones and other quinolones consumption on resistance to fluoroquinolones in invasive *E. coli* from humans was estimated at 0.231, whereas the direct effect of human consumption was assessed at 0.580. The effect of resistance in animals on resistance in humans was significant (conversely to what observed in the PLS-PM model on multiblock manifest variables) but weak (path coefficient = 0.345 and p = 0.03). About 75% of the variance of resistance in humans was explained by these two factors (i.e. consumption in humans and resistance in animals). R^2 for resistance in animals was of the same magnitude as when estimated in the PLS-PM model on multiblock manifest variables (Figure B.6).
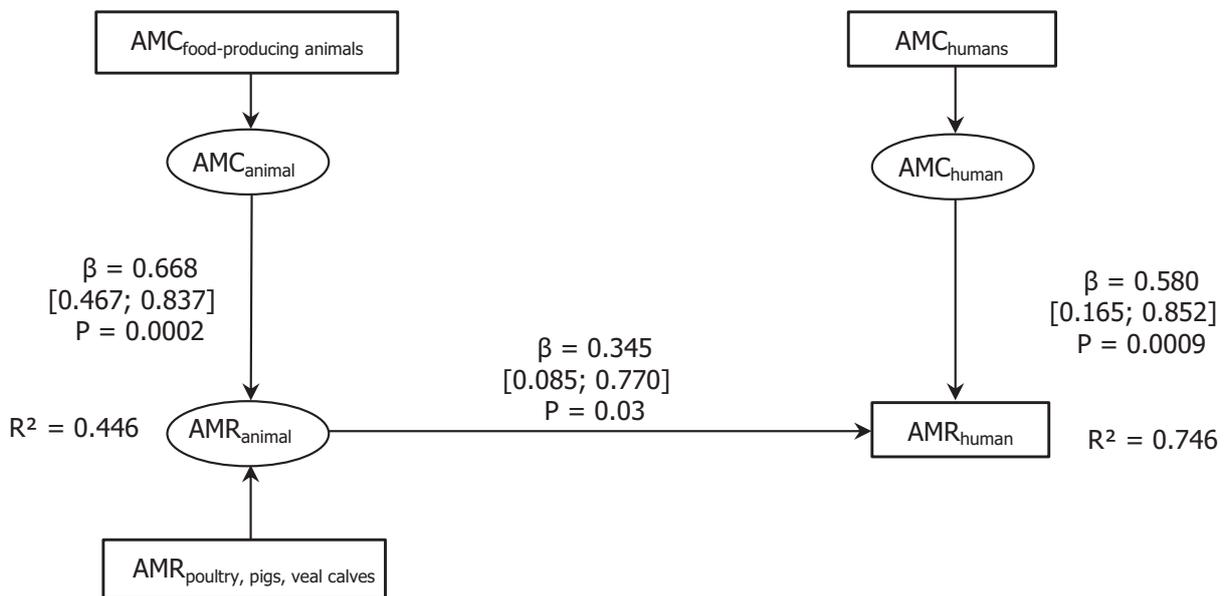
26 countries: AT, BE, BG, CY, CZ, DE, DK, EE, ES, FI, FR, HR, HU, IE, IT, LT, LV, NL, NO, PL, PT, RO, SE, SI, SK, UK.

Figure B.6: Diagram of the PLS-PM of resistance to fluoroquinolones in human invasive *E. coli* (2014 and 2015) considering resistance to fluoroquinolones in indicator *E. coli* from food-producing animals (pigs and veal in 2015 and poultry in 2014), consumption of fluoroquinolones and other quinolones in humans (2014–2015 average, expressed in mg/kg of biomass), in animals (2014–2015 average, food-producing animals, expressed in mg/kg PCU)

Appendix C − Emerging AMR issues since publication of JIACRA I

C.1.   Plasmid-mediated resistance to colistin (see Section 14.3)

In November 2015, transferable resistance to the polymyxin antimicrobial colistin, mediated by the *mcr-1* gene, was reported in *E. coli* from pigs in China and some other countries in the Far East and Europe (Liu et al., 2015). Subsequently, *mcr-1* has been identified in retrospective studies of colistin-resistant *E. coli* and *Salmonella* from many countries worldwide (Skov and Monnet, 2016). More recently, a novel *mcr* gene, *mcr-2*, has been identified in passive screening of porcine and bovine colistin-resistant *E. coli* isolates made in Belgium between 2011 and 2012 that did not show the presence of *mcr-1*. Of particular note is that this *mcr* gene was associated with a highly transmissible IncX4 plasmids (Xavier et al., 2016). ECDC have published a rapid risk assessment of the implications for human medicine of plasmid-mediated colistin resistance (ECDC, 2016b).

In 2016, the EU Antimicrobial Advice ad hoc Expert Group (AMEG) noted that there are wide variations in the use of colistin in EU MSs adjusted for the biomass under exposure (kg livestock, expressed in PCU) between countries (EMA/AMEG, 2016). Countries with intensive livestock production can have a level of use below 1 mg/PCU (e.g. Denmark and the UK) or much higher, up to 20–25 mg/PCU (Italy and Spain). The AMEG have therefore recommended that for the 'high and moderate consumers' the target and desirable levels are set at 5 mg/PCU and 1 or below 1 mg/PCU, respectively, based on the observations on the level of use in other countries. The AMEG further recommended that more information should be gathered to determine the minimum level of colistin use that can be achieved while maintaining animal welfare and preventing the increased use of other CIAs.

More recent studies have demonstrated the presence of the *mcr-1* gene mediated by an IncI2 plasmid in isolates of *E. coli* and *Klebsiella pneumoniae* from sewage from different waste water plants in Spain (Ovejero et al., 2017), in colistin-resistant clinical isolates of *K. pneumoniae* made in Laos and France (Rolain et al., 2016) and *mcr-1.2*, a new *mcr* variant carried on a transferable plasmid from a colistin-resistant KPC carbapenemase-producing *K. pneumoniae* strain of human origin in Italy (Di Pilato et al., 2016). These studies clearly demonstrate the ability of *mcr*-carrying plasmids to become disseminated in potentially highly pathogenic strains in humans and the environment in Europe.

C.2.   The emergence and spread of certain multidrug-resistant salmonella serovars

Salmonella Infantis is one of the most commonly isolated serovars and MDR strains have recently been emerging worldwide. Comparative analyses between pre-emergent and the clonal emergent *S.* Infantis populations demonstrated the fixation of adaptive mutations in the DNA gyrase (*gyrA*) and nitroreductase (*nfsA*) genes, conferring resistance to quinolones and nitrofurans, respectively, and the carriage of an emergent-specific plasmid, designated pESI. This self-transferred episome is a mosaic megaplasmid ($\sim$ 280 kb), which increases bacterial tolerance to environmental mercury (*mer* operon) and oxidative stress, and provides further resistance to tetracyclines, sulfamethoxazole and trimethoprim, most likely due to the presence of *tetRA*, *sulI* and *dfrA* genes, respectively. Moreover, pESI codes for the yersiniabactin siderophore system and two novel chaperone-usher fimbriae. *In vitro* studies established that pESI conjugation into a plasmidless *S.* Infantis strain results in superior biofilm formation, adhesion and invasion into avian and mammalian host cells. *In vivo* mouse infections demonstrated higher pathogenicity and increased intestinal inflammation caused by an *S.* Infantis strain harbouring pESI compared with the plasmidless parental strain (Aviv et al., 2014).

MDR *Salmonella* Kentucky strains such as ST198 (see Appendix A above) have been increasingly isolated from across Europe since 2009. Multiple mutations within chromosomal genes *gyrA* and *parC* are responsible for high-level ciprofloxacin resistance. One of the isolates from Poland was extended spectrum β-lactamase- (ESBL) positive: the strain 1643/2010 carried a conjugative 167,779 bp plasmid of IncA/C family. The sequence analysis revealed that it carried a $bla_{CTX-M-25}$ gene and an integron with another β-lactamase-encoding gene bla_{OXA-21}. This is the first known report of a CTX-M-25 encoding gene both in Poland and in *S.* Kentucky worldwide, as well as in the IncA/C plasmid. Analysis of the integron showed a novel arrangement of gene cassettes – *aacA4*, *aacC-A1* and bla_{OXA-21} where the latter might result from an intergeneric gene transfer. The study confirmed that the *S.* Kentucky population isolated in Poland belongs to global epidemics of high level fluoroquinolone-resistant clone ST198 that can carry rare β-lactamase genes (Wasyl et al., 2015).

The identification in the Netherlands in 2016 of clonal clusters shared by extended spectrum-resistant (ESC) *S.* Heidelberg strains in food-producing animals and poultry meat that can cause human infections (Liakopoulos et al., 2016) underscores the risk for potential zoonotic or foodborne transmission of these strains to humans. Although no human infections linked to these contaminated products have been yet documented in the Netherlands, the risk of potential zoonotic or foodborne transmission of ESC-resistant *S.* Heidelberg strains further highlights the necessity for active surveillance and intervention strategies by public health organisations.

The recent identification in Africa of highly drug-resistant epidemic lineages of *S.* Enteritidis capable of bloodstream invasion (Feasey et al., 2016) is a major cause of international concern. Such strains, which exhibit resistance to almost all commonly used antimicrobials encoded by a plasmid which also carries a repertoire of virulence genes, are not primarily zoonotic and are transmitted by person-to-person spread in the community and hospitals. Nevertheless, it is important to realise that isolates of these organisms have been isolated from travellers returning to EU countries, and as such can potentially influence findings on multiple resistance to antimicrobials such as tetracyclines and other commonly used antimicrobials in cases of *S.* Enteritidis in EU countries.

C.3. The ongoing spread of LA-MRSA in certain high-risk groups of workers in direct contact with live animals and of MRSA in pigs and other species

A zoonotic reservoir in food production animals involving a specific clone, MRSA ST398, which spreads extensively in animals and is found in retail meat has been increasingly noted in several EU countries. As such this poses a potential threat to public health, as people in contact with food production animals are at higher risk of colonisation. The most probable transmission route seems to be by contact (Huang et al., 2014), and dust in intensive animal housing is often contaminated with MRSA ST398 (Broens et al., 2008; Cole et al., 2000).

Although the clonal relationship between MRSA strains of CC398 is clear in livestock and people, this is less obvious in horses. Small companion animals typically share MRSA strains that seem to originate from and exchange with a human reservoir (Catry et al., 2010), as well as harbouring other resistant species of *Staphylococcus* (Cohn and Middleton, 2010). Most isolates from clinically infected animals carry numerous genetic elements related to AMR and virulence genes, and a phi3 prophage encoding immune-modulating proteins that is associated with animal-to-human transmission. Recent findings suggest clonal expansion and dissemination of a new subpopulation of CC398 isolates, responsible for invasive infections in various animals, with a considerable potential to colonise and infect humans; probably greater than that of the original pig/human-adapted CC398 isolates (Van Der Mee-Marquet et al., 2014). Recently, specific clones of MRSA have been reported as circulating between pigs and dairy cattle in Italy, raising the possibility of still wider dissemination of such organisms between species (Feltrin et al., 2016).

LA-MRSA was first reported in pigs in 2005 (Voss et al., 2005). To date, LA-MRSA is only prevalent in certain high-risk groups of workers in direct contact with live animals and the spatial distribution of MRSA genotypes suggests interspecies transmission and colonisation of different populations, communities, animals and their products, although a relevant association between contamination of food products and consumers has not been confirmed. The carrier rate of MRSA is high in food handlers in restaurants in some countries and this can lead to contamination of food (Alsamarai et al., 2015). Nevertheless, the proportion of human MRSA infections that are due to LA-MRSA strains remains low overall in the EU and in most EU MSs.

C.4. Transferable resistance to macrolides in *Campylobacter* spp. from food-producing animals

Resistance to macrolides in *Campylobacter* spp. has generally been the result of mutations in ribosomal RNA or ribosomal proteins and these mutations are thought to have incurred fitness costs, accounting for the low occurrence of erythromycin resistance in many countries (Wang et al., 2014). Ribosomal mutations can confer high-level erythromycin resistance (Gibreel and Taylor, 2006). Transferable resistance to the macrolide erythromycin was first described in *Campylobacter* spp. isolates from food-producing animals (including pigs, chickens and ducks) from China in 2014 (Qin et al., 2014; Wang et al., 2014) and frequently resulted in high level resistance to erythromycin, with MICs recorded at > 512 mg/L. Resistance was conferred by the rRNA methylase gene *erm(B)*, which

can be associated with either chromosomal multidrug resistance islands or transferable plasmids. More recently the transferable *erm(B)* gene has been identified in a *C. coli* isolate from broilers in Spain (Florez-Cuadrado et al., 2016), and this may provide a means whereby macrolide resistance can spread rapidly in *Campylobacte*r spp. in animals in the EU. The situation may be compared to tetracycline resistance, which is frequently plasmid-mediated in *Campylobacter* spp., and is frequently detected in many EU MSs at high levels.

Appendix D – Additional tables

Table D.1: Consumption of antimicrobials in humans and food-producing animals, in tonnes, the estimated biomass of the corresponding populations in 1,000 tonnes and consumption expressed in mg/kg biomass[a] in 26 EU/EEA MSs in 2013[b]

Country	Inclusion of 2013 consumption at the hospital	Consumption in tonnes active substance		Estimated biomass in 1,000 tonnes			Consumption in mg/kg biomass		
		Humans	Animals	Total	Humans[c]	Animals	Total	Humans	Animals
Austria	No	43	55	98	528	957	1,485	81.9	57.2
Belgium	Yes	112	260	372	698	1,657	2,355	161.1	156.6
Bulgaria	Yes	52	47	99	453	401	854	115.2	116.1
Cyprus	Yes	7	48	55	54	113	167	136.6	425.8
Czech Republic	No	64	57	121	650	697	1,347	98.0	82.2
Denmark	Yes	50	108	159	350	2,417	2,767	143.9	44.9
Estonia	Yes	6	9	14	83	121	204	70.9	70.4
Finland	Yes	48	12	60	339	514	854	141.8	22.4
France	Yes	721	681	1,402	4,112	7,165	11,277	175.4	95.0
Germany	No	302	1,532	1,834	4,366	8,526	12,892	69.3	179.7
Hungary	Yes	51	176	227	619	763	1,382	82.5	230.7
Iceland	No	2	1	3	20	115	135	120.3	5.3
Ireland	Yes	44	100	144	287	1,762	2,049	154.9	56.6
Italy	Yes	638	1,318	1,956	3,730	4,372	8,102	170.9	301.6
Latvia	Yes	11	6	17	126	167	294	86.7	37.7
Lithuania	Yes	21	10	31	186	339	525	115.7	29.1
Luxembourg	Yes	5	3	7	32	51	83	151.2	52.1
Netherlands	Yes	53	226	279	975	3,226	4,201	54.6	69.9
Norway	Yes	44	7	50	316	1,789	2,104	138.9	3.7
Poland	No	242	577	819	2,379	3,806	6,185	101.8	151.5
Portugal	Yes	75	179	254	620	958	1,578	120.3	187.2
Slovakia	Yes	45	16	60	338	246	584	131.9	63.1
Slovenia	Yes	14	4	18	129	180	309	108.2	22.4
Spain	No	306	2,202	2,508	2,920	6,944	9,864	104.8	317.1
Sweden	Yes	73	10	83	597	796	1,393	122.1	12.7
United Kingdom	Yes	508	422	930	4007	6799	10,806	126.9	62.1
All[a]		3,809	8,063	11,872	30,434	54,882	85,316	123.8	146.9

(a): Calculated from the exact figures (not rounded as shown).

(b): The estimates presented are crude and must be interpreted with caution. Countries with less than 95% data coverage for community consumption in humans were Germany (85%) and the Netherlands (92%). In those countries, the consumption expressed in tonnes, without correction for population or biomass, will be an underestimate. For further limitations that hamper the comparison of consumption of antimicrobials in humans and animals please see Section 14.

(c): Population covered by data in ESAC-Net.

(d): Population weighted mean.

Table D.2: Range, median and population-weighted average of the consumption of the antimicrobial classes selected for analysis in humans and food-producing animals in 26 EU/EEA MSs in 2013, expressed in mg/kg of estimated body weight and year

Chapter	Antimicrobial class	Humans			Animals		
		Range	Median	Average[a]	Range	Median	Average
6	Third- and fourth-generation cephalosporins	0.11–12.9	1.6	3.8	< 0.01–0.7	0.3	0.3
7	Fluoroquinolones and other quinolones	3.0–17.7	6.5	8.3	0–9.9	1.6	3.4
8	Polymyxins	0–0.1	0.01	0.03	0–27.1	1.3	9.0
9	Macrolides	1.6–16.5	6.7	8.3	0–23.2	5.4	10.8
10	Tetracyclines	0.3–13.6	1.8	3.6	0.1–153.0	27.7	53.9

(a): Population weighted mean.
(b): Spearman's rank correlation coefficient.

Table D.3: Spearman's rank correlations between AMC in humans and in animals, expressed in mg/kg estimated biomass and year

Chapter		ρ[b]	p-value
5	Total consumption	0.11	0.594
6	Third- and fourth- generation cephalosporins	0.27	0.184
7	Fluoroquinolones and other quinolones[a]	0.58	0.002
8	Polymyxins	0.25	0.314
9	Macrolides	0.23	0.260
10	Tetracyclines	−0.34	0.088

(a): Same values for fluoroquinolones only.
(b): Spearman's rank correlation coefficient.