Correlation between veterinary antimicrobial use and antimicrobial resistance in food-producing animals: a report on seven countries

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Objectives: To evaluate correlations between antimicrobial use and the prevalence of resistance in commensal Escherichia coli isolates from pigs, poultry and cattle, using data from publicly available national or international reports from seven European countries.

Methods: The link between the quantities of different classes of antimicrobials administered to food-producing animals per country (expressed in mg/population correction unit) and the prevalence of resistance to the different antimicrobial classes (interpreted by EUCAST epidemiological cut-off values) in E. coli isolates (4831 isolates in total) was assessed by means of polynomial regression analysis and determination of Spearman’s rank correlation coefficient.

Results: A quadratic regression best fitted the antimicrobial use and antimicrobial resistance data. The coefficient of determination was, in decreasing order, 0.99 for fluoroquinolones and amphenicols, 0.94 for third-generation cephalosporins and sulphonamides, 0.93 for aminopenicillins, 0.86 for fluoroquinolones, 0.81 for streptomycin and 0.80 for gentamicin and tetracycline. Spearman’s rank correlation coefficient was 1 for amphenicols, 0.96 for sulphonamides, 0.93 for streptomycin and tetracycline, 0.89 for aminopenicillins, 0.71 for gentamicin and 0.70 for third-generation cephalosporins.

Conclusions: These remarkably high coefficients indicate that, at a national level, the level of use of specific antimicrobials strongly correlates to the level of resistance towards these agents in commensal E. coli isolates in pigs, poultry and cattle. However, data restraints reveal the need for further detail in collection and harmonization of antimicrobial resistance and use data in Europe.

Keywords: antibiotics, surveillance, E. coli

Introduction

Discussion on possible links between antimicrobial use in the production of food animals and the emergence of antimicrobial resistance in animals and potentially also humans dates back more than four decades to the Swann Report.1 Since then, many papers have dealt with this alarming topic.2–6 The transfer of resistance between animals and from animals to humans has been studied extensively7–9 and biological mechanisms of gene transfer between animal and human bacteria have been described.10,11 On the other hand, there are studies that question the link between antimicrobial consumption in animals and the prevalence of resistant isolates in humans.12,13 The WHO lists antimicrobial resistance as a global concern and underlines the importance of trustworthy national surveillance systems.14

In Europe, during the last two decades, several EU member states have made great progress towards monitoring antimicrobial resistance in food-producing animals.15–22 However, only in recent years, after clear calls for the urgent harmonization of resistance monitoring programmes,23–26 are national reports being published that use more or less uniform methodology for antimicrobial susceptibility testing, allowing for better comparison between countries. The scientific guidance of the European Food Safety Authority (EFSA) has likely helped.27 Another step forward was the implementation of epidemiological cut-off values set by EUCAST. In this regard, EFSA stated that from 2013 onwards, reports on the occurrence of antimicrobial resistance in the indicator Escherichia coli should become mandatory and be included in each national surveillance programme of an EU member state.27

Regarding the knowledge of veterinary antimicrobial use in Europe, huge improvement has been made through the activities of the European Surveillance of Veterinary Antimicrobial Consumption (ESVAC). In 2012, the second ESVAC report was published, presenting the results on antimicrobial consumption in animals in...
19 EU/European Economic Area member states plus Switzerland for the year 2010. The report revealed important information on the sales of antimicrobial agents per country and the percentage of sales attributed to each antimicrobial class.

Turnidge and Christiansen stated, regarding antimicrobial use and resistance in human medicine, that obvious correlation is not at all obvious when considered carefully. Several studies measuring correlations between antimicrobial use and resistance in human medicine had a marked variation in data selection and study design, making comparisons difficult. At the European level, a human medicine study correlating mean antimicrobial use in European countries and resistance in Streptococcus pneumoniae showed a positive correlation between both. In veterinary medicine, Asai et al. published a study communicating that the overall use of veterinary antimicrobials appeared to contribute to the appearance of antimicrobial resistance in E. coli isolates from apparently healthy animals in Japan. Given the increased availability and comparability of data on antimicrobial resistance and antimicrobial use in animals, the aim of this study was to evaluate whether, based on publicly available data, correlations between antimicrobial use and antimicrobial resistance in commensal E. coli from various food-producing animals in Europe could be identified.

Materials and methods

Study design

Data selection

National or international reports describing European data on antimicrobial use in animals and/or on antimicrobial resistance in E. coli from apparently healthy food-producing animals were examined. Only reports that collected and analysed the data and presented the results in a comparable manner were used.

Antimicrobial use data

Data on antimicrobial use were obtained from the ESVAC 2012 antimicrobial use report. In this report, the annual sales figures in 20 European countries are reported in absolute values as well as in relation to the animal population present in the country. In those countries, antimicrobials can be administered for treatment and/or prophylaxis of infection in animals but, since 2006, a total ban on the use of antimicrobials for growth promotion has been implemented. The magnitude of the animal population is quantified by means of the population correction unit (PCU). The PCU is a technical unit of measurement based on the estimated weight at treatment of livestock and of slaughtered animals for food-producing animals (poultry, pigs, cattle, small ruminants and rabbits) and horses in the corresponding year. The PCU also corrects for the import and export of animals. One PCU is a representation of 1 kg of different categories of livestock and slaughtered animals. In our study, mg per PCU (mg/PCU) was used as a measurement of antimicrobial use for every veterinary antimicrobial class.

Antimicrobial resistance data

Only countries that provided data on poultry, pigs and cattle from apparently healthy animals originating from studies conducted during 2010–11 and for which ≥10 isolates per animal species were available were included. For cattle, some countries only provided data on adult cattle (Austria and Norway), whereas Sweden and Switzerland only provided data on veal calves and Belgium, Denmark and the Netherlands provided data on both adult cattle and veal calves. Additionally, as instructed by EFSA and also proposed by several scientific reports, only national results provided as quantitative data in the form of MICs were included. Antimicrobial susceptibility results were based on EUCAST epidemiological cut-off values, which determine whether a specific isolate is wild-type in relation to a particular antimicrobial and can be used to describe and quantify biological resistance, regardless of clinical efficacy.

Data preparation and handling

Based on the literature search and the selection criteria, data from seven European countries met the requirements to be included in the study. The data sources were (i) the EPMA and ECDC joint scientific report on antimicrobial resistance in zoonotic and indicator bacteria from humans, animals and food in 2010; (ii) the CODA-CERVA report on zoonotic agents in Belgium (trends and sources 2010–11); (iii) the DANMAP 2011 report (Denmark); (iv) the MARAN 2011 and 2012 report (the Netherlands); (v) the NORM-VET 2011 report (Norway); and (vi) the SVARM 2011 report (Sweden).

Data analysis

The antimicrobial resistance prevalence was measured for each animal category. Exact binomial confidence intervals were calculated (with a 95% confidence level) by means of a computed statistical algorithm. For further statistical analysis, the antimicrobial resistance prevalence data were transformed using arcsin transformation so as to follow bivariate normal distributions more accurately. Subsequently, for each antimicrobial
class studied, the best-fit function describing the link between the use per country (expressed in mg/PCU) and the overall resistance prevalence of *E. coli* isolates for that specific antimicrobial agent was determined and plotted. The coefficient of determination ($R^2$) was used to describe the proportion of variation explained by the function.

Additionally, each country was ranked (from lowest = 1 to highest = 7) in terms of resistance percentages, on the one hand, and use, on the other hand, for every antimicrobial agent studied separately and the correlation was determined by means of Spearman’s rank correlation statistics ($\phi$), a non-parametric measure of statistical dependence between two variables. Finally, the average ranking of every country was also calculated for antimicrobial use (average of use ranks for all antimicrobial classes) and resistance (average of resistance ranks for all antimicrobial agents) and again Spearman’s rank correlation coefficient was determined.

Data manipulations and analysis were performed in Microsoft Excel® 2010 edition and IBM® SPSS Statistics 21.0.

## Results

### Veterinary antimicrobial use in Europe: the 2012 ESVAC report

The results from the seven selected European countries on antimicrobial use are presented graphically in Figure 1 for every antimicrobial class. It can be observed that total antimicrobial use differed between countries. Also, there is huge variation in the used amounts (mg/PCU) of the different antimicrobial classes. For some classes this varied between 0 mg/PCU to 0.54 mg/PCU (third-generation cephalosporins), whereas for other classes this varied between 0.05 mg/PCU up to 74.46 mg/PCU (tetracyclines).

Among the countries, Belgium ranked first for six out of seven antimicrobial classes included. The Netherlands ranked first in tetracycline use.

### Antimicrobial resistance in commensal *E. coli* (data compiled from national reports)

In total, results on 4831 *E. coli* isolates were included. There were 1565 isolates retrieved from poultry, 1308 from pigs, 1086 from cattle and 872 from veal calves.

Huge variations in resistance levels were found both between animal species within countries and between countries within animal species. In general, resistance percentages were highest for poultry isolates followed by veal calves, pigs and cattle (Figure 2).

Between countries, Belgium showed the highest levels of antimicrobial resistance for most antimicrobial agents studied in any animal category. For broilers, exceptions were gentamicin and chloramphenicol (the Netherlands) and ciprofloxacin (Austria). For pigs, exceptions were tetracycline (the Netherlands) and streptomycin (Austria). For cattle, exceptions were gentamicin and tetracycline (the Netherlands). Last, for veal calves, exceptions were cefotaxime and streptomycin (the Netherlands). Between animal species, *E. coli* isolates from broiler chickens showed the highest antimicrobial resistance prevalence for four antimicrobial substances (ampicillin, ciprofloxacin, sulphonamides and cefotaxime). Veal calf isolates were the most resistant to tetracycline, chloramphenicol and gentamicin and isolates from pigs were the most resistant to streptomycin.

### Linking antimicrobial use and antimicrobial resistance

The quadratic regression function produced the highest $R^2$ values for all combinations of use data (mg/PCU) and resistance prevalence (Figure 3). High coefficient of determination values were observed in all studied classes, with 0.99 for fluoroquinolones and amphenicols, 0.94 for third-generation cephalosporins and...
sulphonamides, 0.93 for aminopenicillins, 0.81 for streptomycin and 0.80 for gentamicin and tetracycline.

Each country was ranked (from lowest = 1 to highest = 7) in terms of resistance percentages and use for every antimicrobial agent studied separately and overall. Spearman's rank correlation coefficient was 1 for amphenicols, 0.96 for sulphonamides, 0.93 for streptomycin and tetracycline, 0.89 for aminopenicillins, 0.86 for fluoroquinolones, 0.71 for gentamicin and 0.70 for third-generation cephalosporins (Table 1). The result of the overall ranking is shown in Figure 4.

### Discussion

In this study, we attempted to link antimicrobial use data to antimicrobial resistance data at a national level. In order to do so, we first had to compile the available information on both variables. The observed variation in antimicrobial use among countries is high. Of the seven countries for which sufficient data were available both on use and resistance, the lowest consumption for the antimicrobial classes used in this study was seen in Norway (10.22 mg/PCU), whereas the corresponding figure in Belgium is 146.9 mg/PCU (Figure 1).28 When comparing the average use between the seven countries (62.83 mg/PCU), the Netherlands’ use is double the average use (125.85 mg/PCU) in 2010–11, while Denmark’s use is approximately half the average use (33.23 mg/PCU). Also, among antimicrobial classes, large differences were observed between countries. As early as 2010, Grave et al. concluded that there appears to be wide variation between countries in the use of veterinary antimicrobial agents that cannot be explained by differences in the demographics of animal species.28 Differences in national policies on controlling antimicrobial use, veterinarians’ prescribing and dosing habits, pharmaceutical marketing strategies, animal demographics and specific needs for antimicrobial use in specific countries related to specific diseases may be possible explanations for the observed differences.28

The antimicrobial resistance data also revealed variations between countries, between animal categories and between antimicrobial agents. Quinolones, an antimicrobial class that was introduced later than the other classes and was used extensively in poultry over the last decade,44,52 are now already seriously compromised in broiler chickens. In Austria, resistance to ciprofloxacin is at an alarming point (80%). However, it should be noted that resistance in this paper is interpreted by EUCAST epidemiological cut-off values. The use of epidemiological cut-off values allows comparability among countries, offers the possibility of early detection of emerging resistance and is less subject to differences in opinion, which is often the case with more clinically orientated breakpoints.62 Using the example of fluoroquinolones, the ciprofloxacin resistance prevalence in this study is not indicating a level of antimicrobial activity associated with a high likelihood of therapeutic failure (clinical resistance), but it is referring to the presence of acquired and mutational mechanisms of resistance to the agent (microbiological resistance). Nevertheless, as quinolones are a critically important antimicrobial class for human medicine, special actions should be taken for their use in animals.

In order to link antimicrobial use data to antimicrobial resistance data in veterinary medicine at a supranational level, we made use of publicly available information originating from national and international reports. Using this type of data had the significant advantage that the available information was further explored and no additional expensive sampling and analysis were needed; however, there were also a number of disadvantages in terms of data quality, availability and level of detail.

When antimicrobial consumption is considered, an important data limitation is the fact that the antimicrobial use could not be estimated for each animal species. Silley et al.25 stated that, ideally, for antimicrobial use data to have relevance to resistance development patterns, these data should be recorded on the farm, along with the indication of treatment, the route of administration, the dose and duration of treatment and other relevant
Figure 3. Comparison between antimicrobial use data (expressed in mg/PCU) and antimicrobial resistance prevalence data (arcsin transformed). A quadratic trend line was introduced. The equation used and the $R^2$ value are shown. Data from seven European countries. AM, antimicrobial; PCU, population correction unit.
Where antimicrobial resistance is concerned, *E. coli* was selected as the indicator bacterium for several reasons. First, it is the most used Gram-negative indicator bacterium and therefore relevant data are available; second, the abundance of *E. coli* in animal species and humans makes it one of the most likely vehicles for the spread of resistance genes. The limitation of this selection is of course the fact that the obtained results are only valid for this specific bacterium and cannot be readily extrapolated to other antimicrobial–bacteria combinations. Despite the selection criteria used to include resistance data, large discrepancies between the number of isolates obtained per animal species and per country were encountered (Table S1). Bywater et al. highlighted that for surveillance studies performed at regular time periods, the number of samples is of great interest, to ensure that the total number of samples gives a representative image for the whole population. Thus, one challenge for future studies would be to conduct them using representative populations.

A limitation of the data analysis is that correlations were studied between specific types of resistance and use without taking into account clusters of resistance (multidrug resistance) in one isolate (data not available). As a consequence, the effects of con- and cross-resistance selection could not be estimated. Molecular epidemiology and the characterization of resistance in strains would provide further insight into the complex mechanisms of antimicrobial resistance selection.

Notwithstanding all the above-mentioned limitations of the available data, it was deemed worthwhile to investigate possible correlations between use and resistance at a meta level, to determine whether the important differences in use between countries are in agreement with the reported differences in resistance. The coefficients of determination obtained through the quadratic trend lines were $>0.80$ for all antimicrobial classes, suggesting that data on antimicrobial use are capable of explaining a large part of the variation observed in the resistance data at the national level. For most of the antimicrobial classes, the best-fit curve described an asymptotic form towards a certain maximum, translated into a negative sign of the quadratic part of the function. This suggests that for these antimicrobials, above a certain antimicrobial use threshold, a further increase in antimicrobial use does not result in a further increase in the resistance percentage. Only for third-generation cephalosporins, amphenicols and fluoroquinolones was a positive quadratic term obtained, resulting in an exponential curve suggesting that a small increase in use has a large increase in resistance as a consequence.

Also, high correlations were found between antimicrobial use and antimicrobial resistance. For all antimicrobial agents, Spearman’s rank correlation coefficient exceeded 0.70, indicating that in countries with high use of any of the selected antimicrobials high levels of resistance are also encountered (Table 1, Figure 4).

Although all these observations are consistent with a link between use and resistance selection, we have to bear in mind that these analyses were performed on small datasets (seven data points per antimicrobial class) and therefore some caution is warranted in interpreting them. Moreover, the data did not allow for correction for the presence of clusters of resistance data, such as prevailing disease patterns and incidence. In a recent study, Bondt et al. demonstrated that animal demographics strongly influence antimicrobial use, making overall comparisons based on mg/PCU difficult. In Denmark, detailed per species data are available (VetStat database), whereas for most other countries this is not yet the case. If an advanced tracking system such as VetStat could be established throughout the EU, it would allow comparisons between national antimicrobial resistance prevalence studies in more detail and with higher validity. In accordance, the recent initiative of ESVAC to move to antimicrobial use data collection at the species level is to be applauded.

Another limitation is the use of mg/PCU as a consumption measure. Treatment frequency, using defined daily dose animal (DDDA) or used daily dose animal, is a more refined measure of antimicrobial consumption. Although such data are not presently available at the European level, the decision of ESVAC to prioritize and suggest the use of DDDA and defined cure dose animal in the ESVAC project will surely provide more detailed and accurate data in the future. However, these detailed and more accurate data sources are not available yet and therefore we used what is existing, taking into account the limitations.
present in one strain; therefore, the obtained correlation coefficients might be either over or underestimations of the true relationships between the use of specific antimicrobials and specific resistances. On the other hand, each of the data points in itself is a summary of a lot of information on antimicrobial use and resistance for each country. Looking at the information from such a meta point of view may allow better recognition of the overall relationships. Nonetheless, more research, using the more detailed data that will become available in the near future, should be performed to test whether the results obtained in this study can be confirmed.

Conclusions
The current paper, for the first time, describes the direct correlation of antimicrobial use data to antimicrobial resistance data in veterinary medicine at a supranational level, based on publicly available data sources. The observed limitations in data and the subsequent analysis restraints reveal the need for further detail in collection of antimicrobial use data and the harmonization in resistance data collection in Europe. Despite these limitations, this comparison revealed high correlations for all the antimicrobial classes studied. Bearing in mind that antimicrobial resistance is a global concern, the need for policies promoting lower and more controlled use of antimicrobials is urgent and support for implementation should be provided at a European or, even better, global level.

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Transparency declarations
None to declare.

Supplementary data
Table S1 is available as Supplementary data at JAC online (http://jac.oxfordjournals.org/).

References


