D-1.4. A report of the assessment of risk factors for AMR and AMU. Assessment of ecological and management factors associated with AMR and Antimicrobial usage

Antibiotic Resistance Dynamics (ARDIG): The influence of geographic origin and management systems on resistance gene flows within humans, animals and the environment.

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Content

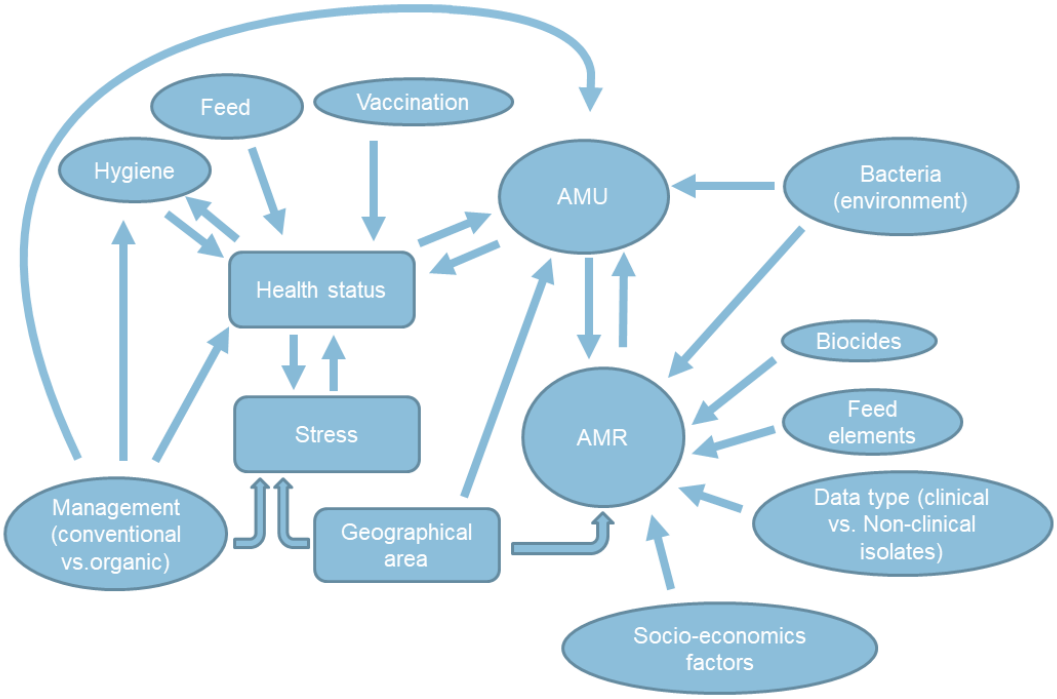
1. Introduction

In previous deliverables (D 1.2. and D 1.3) resistance data of *E. coli* and antimicrobial use data collected from the human and the livestock sector in the ARDIG WP1 between 2014 and 2017 were described. Antimicrobial resistance data from livestock on non-clinical isolates are harmonized in Europe by the Decision 2013/652/EU and by the new Decision 2020/1729/EU that replaces the latter from 1 January 2021. On the other hand, data on clinical isolates are not. Livestock data on AMR in clinical and non-clinical isolates, provided by the United Kingdom, Norway, France and Germany in the WP1 of ARDIG, are based on different laboratory methodologies, different evaluation criteria (i.e. epidemiological vs. clinical), different antimicrobial susceptibility testing (AST) methods (e.g. disc diffusion or broth microdilution) and covering different antimicrobials and animal types. A first approach, performed in previous deliverables, was to transform quantitative resistance data from different laboratory methods and methodologies into qualitative data using specific standards (e.g. The European Committee for Antimicrobial Susceptibility Testing (EUCAST) and the French Society of Microbiology (CASFM)) and evaluation criteria (epidemiological or clinical). However, there will be still an issue with comparability of quantitative data from different methodologies applying this method.A further approach addressed in this delivery is to overcome the lack of AMR harmonization on laboratory methods and methodologies using statistical methods on the quantitative data. This would allow comparing AMR data between and within countries.

2. Factors influencing AMR from the literature

Resistance to antimicrobials is a phenomenon difficult to control as there are many AMR influencing factors. Some of them are described below from the literature: (a) Bacterial exposure to antimicrobials promotes the emergence of resistance as these drugs remove drug-sensitive competitors selecting resistant bacteria. Worldwide actions to tackle high AMR concerning levels are mainly focused on monitoring and reducing AMU in livestock and humans (1, 2). (b) Bacterial status plays an important role in the antimicrobial activity and in the antimicrobial resistance development. Tolerance, as a type of resistance, is defined as the bacteria´s ability to survive in brief exposure to antimicrobials (3). Tolerant strains may not have different MIC values to

those not tolerant (4). There are two types of tolerance: (1) Tolerance by lag: it is a temporary growth arrest (frequently because of stress or starvation of bacteria) and (2) tolerance by slow growth occurring in a stationary phase. Persistence is another resistance type. Tolerance and persistence are similar phenomena occurring at a rate of around 1% (5). While tolerance is referring to the general ability of a bacteria population, persistence refers to the attribute of affecting only a subpopulation of cells (6, 7). (c) Biofilm is a consortium of microorganisms, also bacteria, that create a surrounding matrix formed by proteins, DNA and polysaccharides (8). Biofilm can favour the AMR emergence through three mechanisms: (1) Hinder the spread of antimicrobials in bacterial cells surrounded by extracellular matrix, (2) deteriorate the mismatch repair system or the DNA oxidative repair system and (3) emergence of persistent cells (9). An increase of resistant mutant cells selected under antimicrobial pressure occurs in highly populated biofilms. Matrix with extracellular DNA may also promote the HGT (10). (d) AMR contamination. Most bacteria from humans and animals are commensal being only a small proportion pathogenic. Bacteria are a source of a large number of resistance genes that can be transferred to pathogenic bacteria (11), (e) Some biocides may promote AMR by exposing bacteria to non-lethal concentrations (12). (f) Socio-economic factors have shown to have a significant impact on AMR (13). (g) Some studies describe at the descriptive level an influence of the isolate type (i.e. clinical or non-clinical isolates) on the AMR (14, 15). However no statistical studies had been performed until 2019. The analysis of the differences between clinical and non-clinical isolates would ease the interpretation of those analyses that compare different populations by using different isolate types such as the JIACRA reports (16).



3. Strategies to reduce AMR from the literature

Several strategies have been proposed in the literature to control and reduce AMR:

- Improve awareness and understanding of antimicrobial resistance (17)
- Reduction of AMU in the human and animal sectors (17)
- Restrict as much as possible antimicrobials to therapeutic use in animals. Reduction or prohibition of sub-therapeutic use of antimicrobials as growth promoters or for prophylaxis purposes as much as possible in animal production (Reg (EU) No. 4/2019 on medicated feed)(4, 18).
- Discover new drugs that use new bacteria targets (19). No new antimicrobial with a novel action mechanism has been discovered since 1987.
- Preserve antimicrobials and modify them improving their effectiveness (20).
- Combination of different antimicrobials by the use of different targets on the bacteria (21).
- Avoid poor quality antimicrobials (22).
- Develop new affordable diagnostic tools that provide rapid results with a high sensitivity and specificity (17, 19, 23).
- Increase health status by the use of preventive measures such as vaccination and hygienic procedures (17, 22, 24).
- Genetic selection of livestock resistant to disease (21).

- Promote and support studies on alternative treatments to the use of antimicrobials such as plasmids (25), peptides, phages and vaccines (19).
- Promote organic food consumption (26).
- Digestive microflora replacement via probiotics from humans and animals without AMR genes (19).
- Reduce as much as possible the antimicrobial resistance level in breeding animals (27, 28).
- Limit access to regions with a high resistance prevalence (29).
- Unify government efforts by (i) reducing the risk and uncertainty of antimicrobial clinical trials, (ii) boosting market value for not feeding animals antimicrobials, (iii) strengthen regulation of farm feeding, (iv) assuring the quality of antimicrobials and the prize of new and novel antimicrobials (30).
- Promote pharmacokinetic and pharmacodynamic studies on toxicity and efficacy ranges of antimicrobials in order to provide recommendations for optimal use of drugs (31).
- Collect information by monitoring and surveillance systems about resistance trends and trend changes in order to apply adequate interventions and monitor the impact on them (17).
- Increase the harmonization level between surveillance and monitoring systems of AMU and AMR (22, 32)
- AMR crisis must be addressed from all angles and as a collaborative action between countries (30).

4. Analysis of data collected in the framework of ARDIG WP1

Data from the ARDIG WP1 data collection were analysed. Studies that had already been carried out previously were acknowledged but not repeated (e.g. Comparison between sales data and resistance in non-clinical isolates from livestock across countries (33)). Three novel studies were carried out:

(1) Comparisons between *E. coli* data on clinical and non-clinical isolates from livestock within and between countries.

(2) Comparisons of *E. coli* data on clinical isolates from urinary samples in the human sector and samples from the livestock sector between and within countries.

(3) Comparisons between AMU and data on clinical isolates from livestock between and within countries.

Due to the time constraints associated with the COVID crisis and data limitations, it was decided to postpone comparisons between human and livestock sectors. Typically, national monitoring and surveillance programs include a very limited amount of background data on the isolates, limiting the number of factors that could be analysed in the framework. Therefore, the focus was on five factors for which data were available from several countries in the dataset from ARDIG WP1. These factors were the year of sampling, antimicrobial, antimicrobial use data, animal category and whether the isolate derived from diseased animals (clinical isolate) or from randomly picked isolates from a healthy population (non-clinical isolates). As pointed out before, despite data being available from several countries involved in ARDIG, lack of harmonization was associated with some obstacles to be overcome comparing AMR data on clinical and non-clinical isolates in Germany.

4.1. Analyses of data on AMR in clinical and non-clinical isolates from livestock with a high level of harmonization

Data on clinical and non-clinical isolates collected in the WP1 ARDIG data collection originated from Germany, United Kingdom, France and Norway. Data harmonization issues were encountered as described previously. Therefore, data for the analysis were not ready to be used for analysis across countries. German data on clinical and non-clinical isolates were comparatively well harmonized with seven antimicrobial overlaps (i.e. cefotaxime, ciprofloxacin, colistin, nalidixic acid, tetracycline, gentamicin and ampicillin) in the same animal type and broth microdilution according to ISO 20776-1 as laboratory method to determine minimum inhibitory concentrations of antimicrobials for *E. coli*. This allowed for statistical analysis. We decided to study AMR in broilers and turkeys since poultry and especially the broiler meat sector has increasing relevance as a meat source in Germany and globally (34-37)

This study compared (a) data on AMR in clinical and non-clinical *E. coli* isolates in German broilers and turkeys, (b) investigated AMR changes over time and (c) analysed the associations of changes in AMU with changes in AMR applying univariate and multivariate logistic regression analyses (38). We hypothesised (a) that isolates

from diseased animals might carry higher resistance levels to regular antimicrobial treatments than those from healthy animals and (b) to find an association between AMU and AMR.

Comparison between clinical and non-clinical isolates of *E. coli*

Data on AMR in clinical and non-clinical isolates were compared in this study. Higher resistance proportions were expected in clinical isolates as diseased broilers and turkeys might carry resistance to regular antimicrobial treatments. However, this was only encountered for some animal-drug combinations. Higher resistance proportions in clinical isolates were found to cefotaxime in broilers. In contrast, a higher prevalence of resistance in non-clinical isolates was encountered for ampicillin and colistin in broilers and ampicillin, colistin, gentamicin, and tetracycline in turkeys. These findings suggested that other variables not considered in the analysis may have a significant impact on the resistance prevalence (38).

Associations between AMU and AMR in clinical and non-clinical isolates of *E. coli*

Sales data of antimicrobials to veterinarians in Germany and treatment of broilers and turkeys with antimicrobials decreased from 2014 to 2017 for most drugs(38). Therapy Frequency per animal per time period (TF), is the unit applied in Germany to describe antimicrobial usage in livestock. The study explored the relationship between TF with antimicrobial classes and resistance to drugs from the same class. Associations between AMU and AMR are well-documented (39). However, in this study, this association was only encountered for tetracycline in turkeys and for colistin in broilers suggesting that AMU reduction alone might not be enough in some cases to achieve a decrease in AMR.

Associations between year and AMR

Associations between year and AMR were also only found for colistin and ciprofloxacin for broilers and tetracycline for turkeys. A decreasing association was only observed to colistin for broilers.

4.2. Comparing AMR data on clinical and non-clinical isolates with lack of harmonization on the laboratory methods and procedures in livestock within countries.

Different statistical methods have been proposed in the literature to overcome this issue such as Bayesian models, error rate-bounded and modified error rate-bounded approaches and the Normalized Resistance Interpretation (NRI) method (40-51). All statistical approaches show some subjectivity as a prior agreed standard deviation has to be set (51). EUCAST defines explicitly the NRI method (40, 48) and the ECOFFINDER tool based on (41) as alternative approaches to define the MIC-based ECOFFs (51). The ECOFFINDER tool method only allows estimating MIC-based ECOFFs while the NRI method also allows estimating IZD-based ECOFFs. After discussing within the project group, including the European Joint Action on Antimicrobial Resistance and Healthcare-Associated Infections (EU-JAMRAI), the NRI approach was decided to be applied.

The cut-offs determined from our data by this method were compared to the ECOFFs for the MIC data provided on non-clinical (Norway, the UK, Germany and France) and clinical isolates (Norway and Germany). The result of this comparison revealed that the NRI cut off values are close to those from the gold standard (i.e. EUCAST ECOFFs). The Standard Operating Procedure (SOP) of EUCAST (52) was taken into account for the calculation of these NRI cut-offs as an attempt to reduce the bias as much as possible.

Four countries (i.e. Germany, France, the United Kingdom and Norway) provided data on clinical and non-clinical isolates for livestock in the ARDIG WP1 data collection. However, the comparison of the resistance data from these countries showed a negligible number of overlaps on antimicrobials and animal types. While there was a substantial overlap for the non-clinical isolates based on CID 2013/652/EU and CID 2020/1729/EU, this overlap was limited between the clinical and non-clinical isolates within countries and for the clinical isolates between countries. The resistance comparison between data on clinical and non-clinical isolates from Germany and France showed the largest overlap regarding the antimicrobials tested (i.e. ampicillin, colistin, gentamicin, nalidixic acid and tetracycline) and the animal category (i.e. broiler, turkey and calf). Therefore, resistance data from Germany and France were analysed. Unfortunately, disk diffusion data on colistin resistance have methodological limitations

(53) and therefore had to be excluded from the analysis (54). The lack of AMR harmonization on the laboratory procedure was addressed by applying the NRI method.

Data on AMU were available for France and Germany. However, no approach was found to overcome the lack of harmonization in these data as the units used were not convertible and different populations were studied concerning use in Germany and France. We therefore could not include AMU as an explanatory variable in the statistical analysis across countries. Two AMU units without any direct relationship and reporting on different animal categories were used to compare at the descriptive level the relation between AMU and AMR within each country. It was again expected to see a higher resistance prevalence in clinical isolates per animal class (i.e. broiler, turkey and calf), assuming differences observed in Germany for poultry were an exception.

Two types of logistic regression analyses were performed. The first analysis compared resistance proportions in clinical vs. non-clinical isolates of *E. coli* across the years within each country per animal category and drug while the second analysis included country as a variable. The full analysis included only data from Germany and France. However, some analyses could include data from the UK on broilers. The data set on broilers from Norway was too small to be included in the cross-country analyses. However, they were considered in the discussion(55).

Associations between resistance to a drug and the isolate type (i.e. clinical vs. non-clinical) within countries

A higher prevalence of resistance in the isolates was found in clinical isolates for: (a) calves to ampicillin, gentamicin, tetracycline and nalidixic acid in Germany and France and (b) broilers to gentamicin in France. This was in line with the hypothesis set up in the study. In contrast, lower resistance proportions were encountered in clinical isolates from broilers and turkeys to ampicillin and tetracycline in Germany, France and the UK and to gentamicin in isolates from turkeys in Germany. This suggested that the higher risk of AMR in one isolate type (i.e. clinical or non-clinical isolates) was strongly associated with the relationship between animal species and antimicrobials.

Associations between year and resistance

The study showed a decreasing resistance trend across the years for tetracycline (for broilers, turkeys and calves in France and for turkeys in Germany), nalidixic acid (for calves in France and for turkeys in Germany) and gentamicin (for calves and turkeys in France and for calves in Germany) suggesting that measures carried out against AMR in each country have effective results. Only resistance to nalidixic acid in isolates from broilers in France showed an increasing trend.

Differences in resistance proportions between countries

Higher resistance levels to ampicillin for calves and to tetracycline for broilers, turkeys and calves were encountered in France. In contrast, a higher risk of resistance to nalidixic acid in isolates from broilers, turkeys and calves and to gentamicin in isolates from calves and turkeys was observed in Germany. This is in line with differences in antimicrobial use data, although the comparison of these data has many caveats.

5. Potential sources of bias in both studies

Both studies showed similar biased sources. The sampling frames from data on clinical and non-clinical isolates differ being able to contribute to the differences encountered in this work. Data on non-clinical and clinical isolates compared in this work differed respectively in the following aspects: (a) Mandatory (non-clinical) vs. voluntary (clinical isolates) data collection basis, (b) isolate collection at the slaughterhouse vs. during the lifetime, at time of death or during post mortem, (c) isolate collection at a fixed age vs. different ages, (d) caecal samples vs. diverse sample origins and (e) data representative for the animal population in the country vs. data representative for the samples examined in the laboratories contributing to the system.

Further, in the second study, NRI cut-offs were generated and used for the data interpretation trying to make the best use of available data. EUCAST has a defined Standard Operating Procedure (SOP) for doing these calculations, but with the available data, we could only do the calculations violating this SOP with respect to the number of laboratories and isolates to be included (56). Therefore, NRI cut-offs calculated cannot claim to be fully accurate. However, doing the calculations on

different sub-sets of the data produced very similar results, which encouraged us to proceed.

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