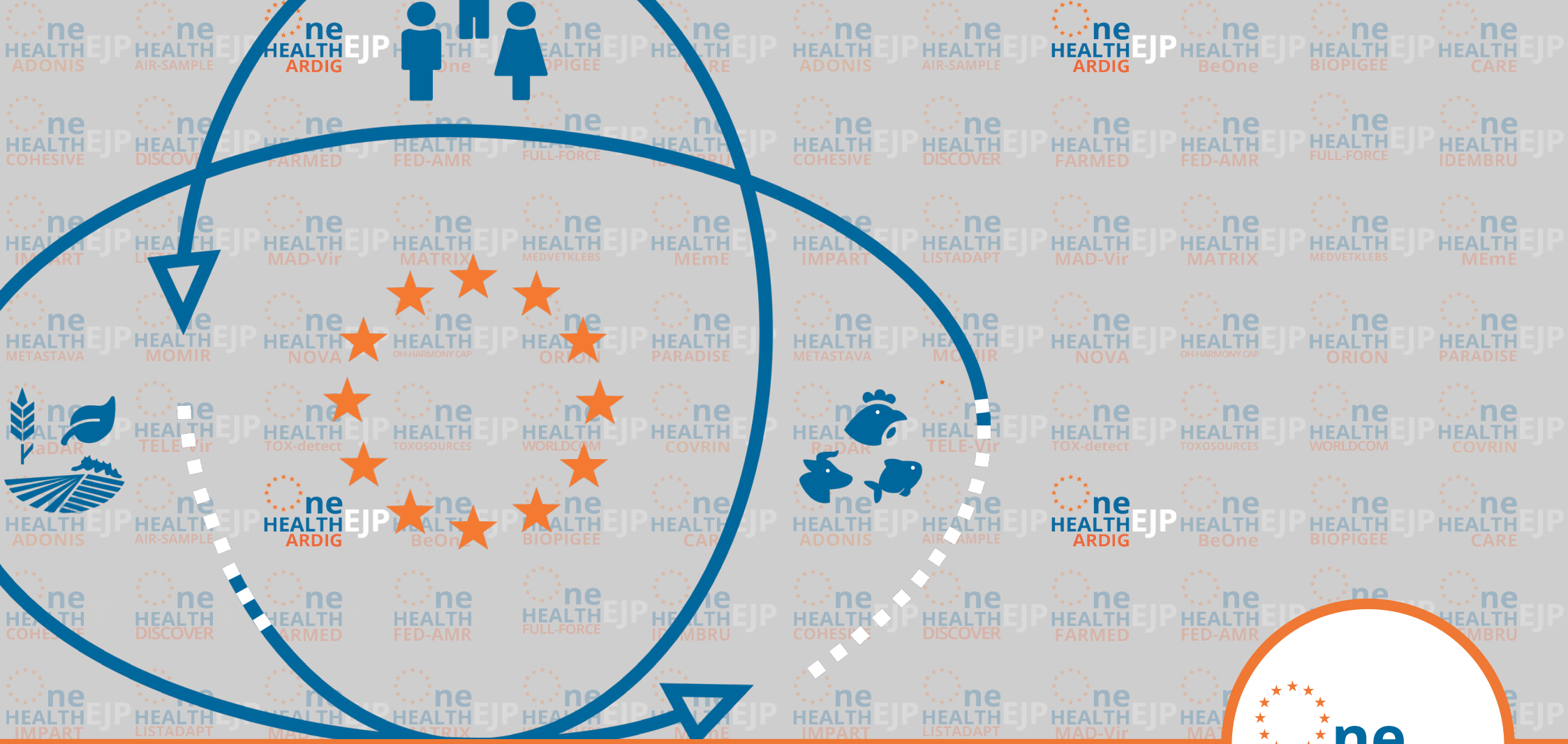

OHEJP PROJECT OUTCOMES

ARDIG

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

May 2023

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WHAT IS ANTIBIOTIC RESISTANCE DYNAMICS? HOW IS THE FLOW OF RESISTANCE GENES WITHIN HUMANS, ANIMALS AND THE ENVIRONMENT INFLUENCED?

Antibiotic resistance dynamics describes the growth or change of antimicrobial resistance (AMR) within an epidemiological unit, such as in humans, animals, a food source, or the environment (Singh *et al.*, 2021).

AMR is a global issue, which has continued to rise, due to the indiscriminate use of antibiotics in agriculture and both human and veterinary medicine (WHO: Antimicrobial resistance, key facts). Due to this, the food chain and the environment have also been affected, resulting in the selection and increased prevalence of antimicrobial resistant bacteria. Despite heightened awareness, AMR is still a significant global burden and is responsible for an estimated 75,000 human fatalities a year (Singh *et al.*, 2021) and increased morbidity has led to extended hospital admissions. Reports of multidrug-resistance bacteria are now common and clinicians, in both human and veterinary medicine, are now resorting to the use of last-line antibiotics, such as colistin (Nation and Li, 2010), or unorthodox antimicrobial combinations, for the treatment of antibiotic-resistant bacterial infections. Due to globalisation, the dissemination of multidrug-resistant bacteria can occur quickly, increasing the likelihood in the flow of antibiotic resistance genes between countries (Berndtson, 2020). If not addressed, the AMR issue is projected to cause 10 million deaths annually and costs 100 trillion US Dollars by 2050 (O'Neill, 2016).

Understanding the dynamics of AMR is crucial, in order to develop improved methodologies for surveillance and gain further insight into routes and mechanisms of transmission. However, due to the complexity in the dynamics of AMR, a One Health approach (One Health Commission: What is One Health?) must be undertaken as AMR in humans, animals and the environment are inherently linked (Singh *et al.*, 2021).

Geographic origin can influence the flow of antibiotic resistance genes, as methodologies of detection, national surveillance, interpretation, and approaches to stewardship can vary between countries (Antimicrobial Resistance Collaborators, 2022). This can be due to differences in government policy, regulation of antibiotics and variations in the treatment of infections between human and animal clinicians. If progress is to be made in the fight against AMR, harmonisation of these systems both nationally and internationally is required (Michael, Dominey-Howes and Labbate, 2014). By introducing standardised practices, data on the flow of resistance genes in various geographic locations, can be more rapidly disseminated and interpreted, allowing for greater outcomes in limiting the future spread of AMR.

Using its unique position, the One Health EJP (OHEJP) is able to facilitate a cooperative approach between institutes, an enable the delivery of important multidisciplinary research to attain optimal health and wellbeing outcomes for humans, animals, and the environment.

Antibiotic resistance through overuse

Examining the flow and location of resistant genes

WHAT IS THE ARDIG PROJECT?

The OHEJP ARDIG project involves 10 partners across Europe: the Animal and Plant Health Agency (APHA), the University of Surrey (UoS), and Public Health England (PHE) in the UK, the Federal Institute for Risk Assessment (BfR) and the Robert Koch Institute (RKI) in Germany, the Norwegian Veterinary institute (NVI) in Norway, the Institute Pasteur (IP) and the French Agency for Food, Environmental and Occupational Health & Safety (ANSES) in France, the Complutense University (CU) in Spain and Wageningen Bioveterinary Research institute (WBVR) in the Netherlands.

Through this multidisciplinary partnership, the ARDIG project was designed to examine the dynamics of antimicrobial resistance in the epidemiological units of humans, animals, food, and the environment. The six European countries involved, represented unique climate and management systems, each with the potential for transmission of antimicrobial resistance.

At the start of the project, several important knowledge gaps were identified, including:

- Substantial lack of harmonisation in AMR and antimicrobial usage (AMU) data between sectors within and across countries
- AMR differences between clinical and non-clinical isolates i.e. diseased vs healthy animals
- AMR and persistence in bacterial strains in both humans and animals
- Lack of harmonisation in methodologies for AMR gene prediction.

The ARDIG project aimed to address these knowledge gaps by:

- Comparing AMR and antibiotic sales/usage with existing national surveillance and research programs in order to harmonise the human and veterinary sectors
- Undertaking longitudinal studies of AMR persistence in isolates from hospitals and livestock
- Characterising AMR, transmission of plasmids and fitness of multidrug-resistant isolates; in order to harmonise *in silico* AMR gene prediction and assess the impact of methodology on AMR gene prediction.

ARDIG PROJECT OUTCOMES

The ARDIG project aimed to harmonise the Antimicrobial usage (AMU) and Antimicrobial resistance (AMR) data for comparison, as well as study of AMR persistence and transmission.

Comparing existing AMU and AMR data.

The OHEJP ARDIG project identified a lack of harmonisation on AMU and AMR in the livestock sector and on AMR in the human sector. However, some overlap between national and international systems was observed. A One Health approach requires harmonisation in all aspects of AMR and AMU, between systems in the human, animal, and food sector. ARDIG provided suggestions to address this, including the standardisation of data type collected in areas such as antimicrobials tested, laboratory methods, choice of antibiotic breakpoints and analysis. Differences in AMR between clinical and non-clinical isolates were identified within and between countries for different animal populations. Higher resistance levels in clinical isolates than in non-clinical isolates were found for calves, while the opposite was found in isolates from broilers and turkeys. Decreasing resistance was found in animal isolates between 2014 and 2017. For more details about these studies, read the following ARDIG deliverables' reports: D1.1: A report of AMR and AMU data in livestock and humans in the six participating countries, and with indication to its quality, comparability and purpose, and D1.2: Description of the specified AMR prevalence/frequency and AMU at population/country/regional level, and this research publication. The results suggest that measures put in place to combat AMR, including the reduction in AMU in each country, have been effective. A workshop held with experts in the field, including those from EFSA, EMA and ECDC and other European projects, allowed recommendations from the ARDIG project, to improve "One Health" surveillance strategies.

Longitudinal studies of AMR persistence.

Isolates of *E. coli* were collected from retrospective and prospective longitudinal studies, including *E. coli* isolated from urinary tract infections from local hospitals and livestock (cattle, pigs, and poultry). In addition, *E. coli* isolated through EU harmonised surveillance for AMR were also characterised. Whole genome sequencing (WGS) was undertaken and a total of 450 genome sequences were obtained. Analysis was carried out by five pipelines to establish their AMR profiles: APHA SeqFinder/Abricate, PHE GeneFinder, WBVR, Ariba, ResFinder/PointFinder. The AMR genotypes were compared with the corresponding phenotypes obtained using Antimicrobial susceptibility tests for these isolates. Whole genome sequences of over 3000 isolates collected from longitudinal, as well as national surveillance by partners were also compared. Overall, bacterial strains isolated from humans shared greater similarities with each other, than with those isolated from animals. Isolates from animals also shared greater genetic similarities with each other, than with isolates from humans. Other key discoveries from this deliverable, include the importance of using both WGS and antibiotic selective media when monitoring AMR, and although carbapenemase-producing *E. coli* have disseminated globally, their selection is a multi-step process.

For more information, please read the following publications: Nunez *et al*, 2022; Storey *et al*, 2022; Savin *et al*, 2021; Massot *et al*, 2020; Patino-Navarrete *et al*, 2020; Gay et al, 2019.

AMR characterisation, transmission of plasmids and fitness of multi-drug resistant (MDR) isolates.

The AMR pipeline comparison aimed to help harmonise *in silico* AMR gene prediction and to assess the impact of methodology on AMR gene prediction. Using collected isolates, AMR gene, plasmid and mobile genetic element characterisation were performed, using WGS as well as other molecular techniques. Phylogenetic analysis and AMR profiling performed by members of the ARDIG project, have helped identify possible transmission events or epidemiological links between different compartments and countries. A novel bioinformatic approach was developed to determine similarity of IncI circulating plasmids present in isolates across compartments. This provided an important overview into the types of AMR plasmids commonly circulating, which include following types: IncI, IncF and IncX.

To know more about these results, please read the following publications: Getino *et al*, 2022; Thomson *et al*, 2022; Juraschek *et al*, 2021; Brouwer *et al*, 2020; Duggett *et al*, 2020; Rodriguez-Rubio *et al*, 2020; Brouwer *et al*, 2019.

SCIENTIFIC PUBLICATIONS

The OHEJP ARDIG project worked towards the development of harmonised and innovative methods to monitor and further investigate AMR. The scientific outputs from this project, will help provide a better understanding of the drivers of AMR transmission and its consequences across these regions, allowing it to be studied more widely in future.

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MEET THE TEAM

Each of the One Health EJP projects creates a unique Europewide network of institutes with wide-ranging expertise to achieve objectives using a cohesive One Health approach. We bring together scientific communities with expertise in medical, veterinary, and environmental health, and use integrated approaches to solve complex global issues.

The OHEJP ARDIG project worked collaboratively to achieve its aims and to produce impactful scientific outcomes, which may benefit scientists and policymakers worldwide.

The One Health EJP members of this project were from the following institutes:

- Institut Pasteur (IP), France
  Dr. Philippe Glaser
- French Agency for Food, Environmental and Occupational Health & Safety (ANSES), France
  Dr. Jean-Yves Madec
  Dr. Marisa Haenni
- Federal Institute for Risk Assessment (BfR), Germany
  Dr. Bernd-Alois Tenhagen
  Dr. Jens A. Hammerl
- Robert Koch Institute (RKI), Germany
  Dr. Tim Eckmanns
  Dr. Sebastian Haller
- Wageningen Bioveterinary Research institute (WBVR), Netherlands
  Dr. Michael Brouwer
- Norwegian Veterinary institute (NVI), Norway
  Dr. Marianne Sunde
- Complutense University (CU), Spain
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  Dr. Francesca Martelli
- Public Health England (PHE), UK
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  Dr. Matthew Ellington
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