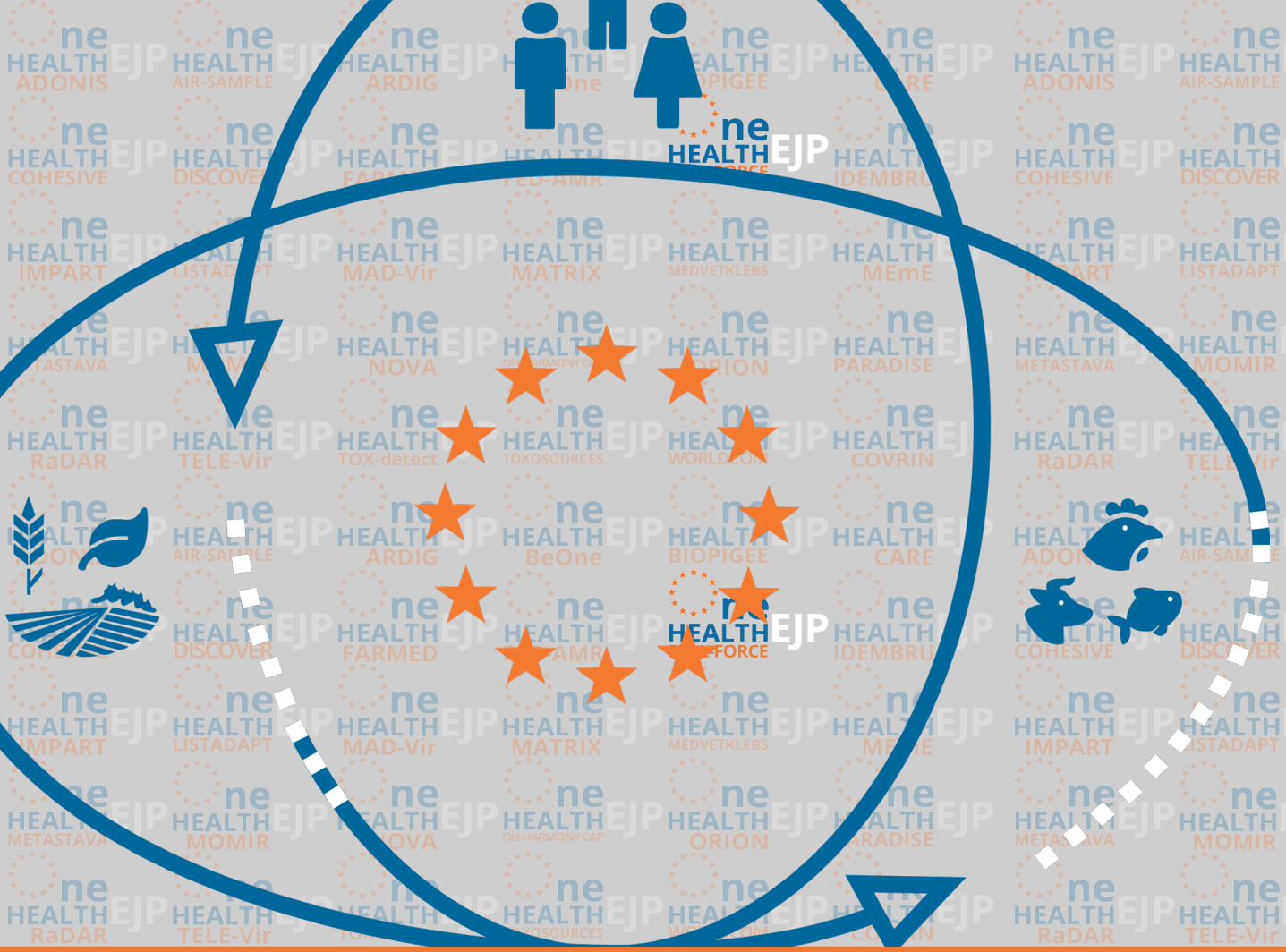

OHEJP PROJECT OUTCOMES

FULL-FORCE

Full-length sequencing for an enhanced EFFORT to map and understand drivers and reservoirs of antimicrobial resistance

April 2023

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WHAT IS ANTIMICROBIAL RESISTANCE SURVEILLANCE AND WHY ARE MOBILE GENETIC ELEMENTS IMPORTANT?

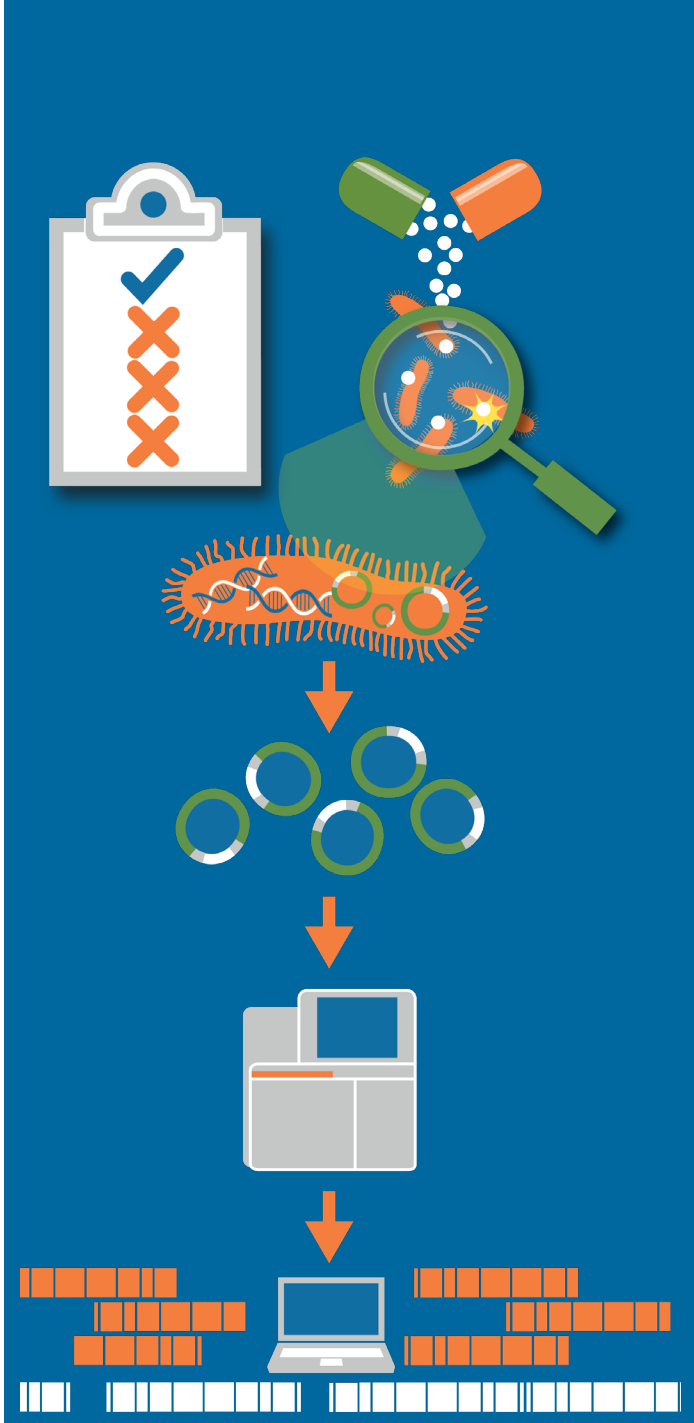
Antimicrobial resistance (AMR) surveillance detects and records the trends and changes in drug-resistant microorganisms and resistance determinants such as AMR genes (fao.org/antimicrobial-resistance/key-sectors/surveillance-and-monitoring/en). AMR surveillance data improve the understanding of the complex epidemiology of AMR.Therefore it is an essential governmental tool to develop empirical therapeutic guidelines, to design intervention and infection control policies, and to direct research agendas (Fuhrmeister and Jones, 2019).

However, current surveillance practices fall short in grasping the two distinct mechanisms of the continuous flux of AMR genes: clonal (bacterial reproduction) versus horizontal transmission, across species and reservoirs. Horizontal gene transfer allows micro-organisms to exchange segments of DNA, called mobile genetics elements, and consensus is growing that it is an important driver of AMR (Sun D., Jeannot K. *et al*, 2019). Current standard application of whole genome sequence (WGS) data for epidemiological surveillance is typically restricted to variation within core chromosomal loci (specific physical location of a gene or other DNA sequence on a chromosome); meaning that plasmids (small circular DNA molecule separate from chromosomal DNA that replicates independently) and other mobile genetic elements (segments of DNA mediating the movement of DNA within genomes or between bacterial cells) are mostly overlooked (Partridge *et al*., 2018).

Most of the research in veterinary and public health laboratories use sequencing platforms that provide short sequence reads as outcomes (Harris *et al*., 2021), making it difficult to correctly perform the reconstruction of large plasmids and transposons (sequences of DNA that move within a genome, from one location to another) using contemporary tools. This complexity can often be resolved by long sequence reads generated with Single Molecule, Real-Time (SMRT) sequencing technology (PacBIO, MinION or similar) (Amarasinghe *et al*., 2020). Although several veterinary and public health laboratories had strong interest in implementing this technology, many struggled with its technical implementation, resulting in a wide knowledge gap between (and even within) countries.

Consequently, there was limited knowledge on which mobile genetic elements are currently in circulation, which ones are a threat, and how clinicians and public health workers can manage the crisis of antibiotic resistance. Therefore, a harmonised technological revolution in sequencing was required.

The One Health EJP uses its unique position to facilitate a collaborative approach between institutes to deliver important multisectoral research, and attain optimal health and wellbeing outcomes for humans, animals, and the environment. We bring together 43 acclaimed European scientific institutes and the MedVetNet Association working together on 47 research projects to address potential and existing risks that originate at the animal-human -environment interface.



WHAT IS THE FULL-FORCE PROJECT?

The FULL-FORCE project involves 16 OHEJP partners across Europe: the French Agency for Food, Environmental and Occupational Health & Safety (ANSES) and the French National Research Institute for Agriculture, Food and Environment (INRAe) in France, the National Health Institute Dr. Ricardo Jorge (INSA) in Portugal, the Istituto Superiore di Sanità (ISS) and Istituto Zooprofilattico Sperimentale del Lazio e Toscana (IZSLT) in Italy, the Animal Plant Health Protection Agency (APHA) in the UK, Sciensano in Belgium, the National Institute for Public Health and Environment (RIVM) and Wageningen Bioveterinary Research (WbvR) in The Netherlands, the German Federal Institute for Risk Assessment (BfR) in Germany, the National Veterinary Research Institute (PIWet) in Poland, the Statens Serum Institut (SSI) and DTU National Food Institute (DTU) in Denmark, the Swedish National Veterinary Institute (SVA) and the Public Health Agency of Sweden (FoHM) in Sweden, and the Norwegian Veterinary Institute (NVI) in Norway. A collaboration was also established with the Netherlands Centre for One Health (NCOH) at Utrecht University.

With these partnerships between human, animal and plant health, food safety, and environmental protection institutes, the FULL-FORCE project was designed to supply EU partners with a technological toolbox and training in Single Molecule, Real-Time sequencing. This allowed a paramount step for the effective integration of Mobile Genetic Elements (MGE) typing in One Health AMR surveillance, whilst delivering insight in dominant MGEs which are driving resistance among commensal and pathogenic Enterobacteriaceae in Europe.

The current surveillance systems fail to:

- Define plasmid types;
- Detect and monitor emergent high-risk plasmids that contain multiple resistance genes;
- Identify 'plasmid outbreaks' in healthcare or other settings;
- Determine how AMR plasmids transmit across complex one-health landscapes, and the associated risks to human and animal health.

The FULL-FORCE project aimed to address these knowledge gaps by:

- Delivering to the consortium wet lab protocols, for the creation of long-read sequencing dataset;
- Constructing and benchmarking a common and open source bioinformatic pipeline (informatic collection and analysis of biological data and information), allowing the harmonisation of bioinformatic skills needed for plasmid sequencing;
- Applying this methodology on a wide variety of study cases, such as:
  - The *K. pneumoniae* plasmidome, in the context of resistance to carbapenems, which are the most effective antibiotics against complicated infections caused by AMR bacteria;
  - The pESI megaplasmid, causing AMR in *Salmonella enterica* serovar Infantis throughout Europe;
  - The IncZ plasmids;
  - The Extended-spectrum beta-lactamase (ESBL) plasmids in *E. coli* isolated from horses;
  - The transmission of AMR in among broiler chicken.

FULL-FORCE PROJECT OUTCOMES

The FULL-FORCE project aimed to improve the surveillance of mobile genetic elements (MGEs)-mediated AMR by introducing long-read sequencing methodology. It enabled harmonisation of plasmid sequencing capacity across 17 European public health and veterinary institutes and provided an unprecedented amount of open access data on fully sequenced bacterial strains, which can be used in follow-up studies. It also prepared public health institutes for an 'AMR surveillance 2.0' based on plasmid data, and by doing so, it paved the way for detailed surveillance of AMR transmission in the next decade.

One of the main outcomes of the FULL-FORCE project is the construction and benchmarking of a standardised pipeline for automated analyses of long-read data, and plasmid assembly: the Full Force Plasmid Assembler (FFPA). This was created by Henrik Hasman's team, at SSI. This pipeline can be readily implemented and allows public health and veterinary labs to proceed locally with plasmid assembly and study. This tool, that would allow the harmonisation of plasmids and other MGEs surveillance, can be accessed with this link.

This project also led to the discovery of the importance of IncZ plasmids in AMR horizontal transmission. The first IncZ plasmid was described in 1983. Later, seven additional distinct IncZ plasmid groups have been described. However, the most commonly used *in silico* plasmid typing scheme does not discriminate between the different plasmids within the IncK/B/O/Z group. The FULL-FORCE project's methodology revealed that IncZ is the most observed type within the IncK/B/O/Z plasmid group with a prevalence of 52%. Moreover, they discovered that a particular IncZ plasmid type is more confined to bacteria isolated from sick patients. The particular features of this plasmid and why it is causing more disease is still under investigation.

The FULL-FORCE methodology was also used to study the global distribution and diversity of prevalent plasmids present in sewage water. It uncovered a high degree of plasmid diversity in sewage samples from 24 geographic locations and highlighted antimicrobial resistance genes, particularly macrolide resistance genes, as abundant in sewage water plasmids found in Firmicutes and *Acinetobacter* hosts. Macrolides are broad-spectrum antibiotics which are used in both the human and veterinary sector. The emergence of macrolide resistance in these bacteria suggests that macrolide selective pressure exists in sewage water and that the resident bacteria can readily acquire macrolide resistance via small plasmids. Plasmids hosted by the bacterial genus *Acinetobacter* were predominant among the European sewage water. Results suggested a prevalence of plasmid-backbone gene combinations over others. This could be related to particular bacterial genera that act as bacterial hosts. These combinations also reflected the geographical locations of the sewage samples. This highlights a potential global reservoir of antibiotic resistance in these pathogens. Learn more by reading the following research publications: journals.asm.org/doi/10.1128/msystems.00191-22 and doi.org/10.1128/mSystems.00283-21

This project also studied the transmission dynamics of AMR within a chicken farm, using two different types of transmission models: one based on the immune development of chickens, and one based on the infection characteristics of the bacteria. Both included the environmental contamination effect between production rounds and within flocks and were based on two different assumptions, which were: development of immunity of the chickens and difference between the infection characteristics of the bacteria. Both models were able to describe

the observed transmission dynamics within and between the production stages and quantitatively estimate the outcome of the interventions. Both models were also able to indicate the most effective intervention for improving farm management and eliminate bacteria from the farm environment. For more information, have a look at the conference poster.

The increasing prevalence of multi-drug resistant *Salmonella enterica* serovar Infantis (causing salmonellosis) in Europe is a cause of major concern. The multidrug resistance determinants are generally harboured on a large plasmid named 'plasmid of emerging *S. enterica* Infantis': pESI. The consortium was able to show that although these plasmids look similar, they display differences in AMR virulence genes depending on their origin. Subsequently, the FULL-FORCE methodology allowed a more detailed surveillance of this megaplasmid. The results of this study are published in a research paper.

During the FULL-FORCE project, whole-genome sequencing was performed on seventy-one *E. coli* isolates originating from two poultry and two pig slaughterhouses in Germany, and expressing high rates of multidrug-resistance. It was discovered that these isolates constituted a reservoir for 53 different antimicrobial resistance determinants, including high-risk clones involved in human infections worldwide. An extraintestinal pathogenic *E. coli* pathotype was detected in 17.1% and 5.6% of the isolates from poultry and pig slaughterhouses, respectively. Uropathogenic *E. coli* (UPEC) was also discovered in the effluent of an in-house wastewater treatment plant (WWTP) of a poultry slaughterhouse, facilitating their further dissemination into surface waters. Because these bacterial have been shown to be pathogenic for humans, the use of strict hygiene measures is required at slaughterhouses to prevent exposure to employees and spill-over in the general population. Read more in the published research article.

Thanks to its innovative methodologies, the FULL-FORCE project led to new discoveries in the field of AMR detection and surveillance. When undertaking in depth characterisation of qnrS1-carrying plasmids using WGS with NextSeq, PacBio and Oxford Nanopore, it was discovered that assembly of long-read sequences are error prone and can yield in a loss of small plasmid genomes. In contrast, short-read sequencing was shown to be insufficient for the prediction of a linkage of AMR genes to specific plasmid sequences. It was the combination of both methodologies, termed hybrid sequencing, which led to the most reliable approach for AMR typing and risk assessment. As two final examples, the FULL-FORCE project uncovered (1) the first evidence in Europe of an MDR, bla_{NDM-4}-positive *Escherichia coli* isolated from a food-producing animal, harboured by a novel IncFII plasmid. https://doi.org/10.1093/jac/dkaa374, and (2) A carbapenem-resistant isolate of *Enterobacter cloacae* complex that was also discovered and identified as *Enterobacter asburiae*. Learn more by reading the research publication.

SCIENTIFIC PUBLICATIONS

Teudt, F., Otani, S., Aarestrup, F. M. (2022). Global Distribution and Diversity of Prevalent Sewage Water Plasmidomes. *mSystems*. 7(5), e0019122. DOI: https://doi.org/10.1128/msystems.00191-22

Börjesson, S., Brouwer, M. S. M., Östlund, E., Eriksson, J., Elving, J., Lindsjö, O. K. and Engblom, L. I. (2022). Detection of an IMI-2 carbapenemase-producing *Enterobacter asburiae* at a Swedish feed mill. *Frontiers in Microbiology*. 13, 993454. DOI: https://doi.org/10.3389/fmicb.2022.993454

Furusawa, M., Widgren, S., Evers, E.G., Fischer, E.A.J. (2022). Transmission models of ESBL-producing *E. coli* in the broiler production chain. *Poster presentation*. Society for Veterinary Epidemiology and Preventative Medicine Conference, Belfast, UK. 23-25 March 2022. DOI: https://doi.org/10.5281/zenodo.6581036

Alba P, Carfora V, Feltrin F, Diaconu EL, Sorbara L, Dell'Aira E, Cerci T, Ianzano A, Donati V, Franco A, Battisti A. Evidence of structural rearrangements in ESBL-positive pESI(like) megaplasmids of S. Infantis. *FEMS Microbiol Lett*. 2023 Jan 17;370:fnad014. DOI: https://doi.org/10.1093/femsle/fnad014

Kirstahler, P., Teudt, F., Otani, S., Aarestrup, F. M., Pamp, S. J., (2021). A Peek into the Plasmidome of Global Sewage. *mSystems*. 6, e00283-21. DOI: https://doi.org/10.1128/mSystems.00283-21

Savin, M., Bierbaum, G., Kreyenschmidt, J., Sib, E., Schmoger, S., Käsbohrer, A., Hammerl, J-A. (2021). Clinically Relevant Escherichia coli Isolates from Process Waters and Wastewater of Poultry and Pig Slaughterhouses in Germany. Microorganisms. 9, 698. DOI: https://doi.org/10.3390/microorganisms9040698

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Diaconu, E. L., Carfora, V., Alba, P., Di Matteo, P., Stravino, F., Buccella, C., Dell'Aira, E., Onorati, R., Sorbara, L., Battisti, A., Franco, A. (2020). Novel IncFII plasmid harbouring bla_{NDM-4} in a carbapenem-resistant *Escherichia coli* of pig origin, Italy. *Journal of Antimicrobial Chemotherapy*. 75(12), 3475–3479. DOI: https://doi.org/10.1093/jac/dkaa374

Image: libreshot

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 FULL-FORCE 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:

- Pieter-Jan Ceyssens, Sciensano, Belgium
- Marisa Haenni, French Agency for Food, Environmental and Occupational Health & Safety (ANSES), France
- Manuela Caniça and Vera Manageiro, National Health Institute Dr. Ricardo Jorge (INSA), Portugal
- Jens-Andre Hammerl, Annemarie Käsbohrer, German Federal Institute for Risk Assessment (BfR), Germany
- Saria Otaki, National Food Institute (DTU), Denmark
- Muna Anjum, Animal Plant Health Protection Agency (APHA), UK
- Benoit Doublet, French National Research Institute for Agriculture, Food and Environment (INRAe), France
- Laura Villa, Istituto Superiore di Sanità (ISS), Italy
- Magdalena Zajac, National Veterinary Research Institute (PIWet), Poland
- Stefan Börjesson, Public Health Agency of Sweden (FoHM), Sweden
- Antoni Hendrickx, Fabian Landman, Joost Hordijk, and Marta Rozwandowicz, National Institute for Public Health and Environment (RIVM), The Netherlands
- Henrik Hasman, Statens Serum Institut (SSI), Denmark
- Stefan Widgren, Robert Söderlund, Swedish National Veterinary Institute (SVA), Sweden
- Michael Brouwer, Wageningen Bioveterinary Research (WbvR), The Netherlands
- Jannice Schau Slettemeås, Norwegian Veterinary Institute (NVI), Norway
- Antonio Battisti, Alessia Franco, Patricia Alba, and Virginia Carfora, Istituto Zooprofilattico Sperimentale del Lazio e Toscana (IZSLT), Italy

Project partner from outside of the One Health EJP:

- Aldert Zomer, Netherlands Centre for One Health (NCOH) at Utrecht University, The Netherlands.