IMPART

Improving Phenotypic Antimicrobial Resistance Testing

One HEALTH EJP

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# WHAT IS ANTIMICROBIAL RESISTANCE AND WHY IS IT A ONE HEALTH ISSUE?

Antimicrobial Resistance (AMR) is a global One Health problem and has been recognised by the World Health Organisation (WHO) as "one of the most urgent health threats of our time" (WHO – Antimicrobial resistance Key facts). Globally, AMR microorganisms are currently causing hundreds of thousands of deaths per year and costing billions of US Dollars to healthcare systems (Wellcome - The Global Response to AMR, 2020). If not addressed, this issue is projected to cause 10 million deaths annually and costs 100 trillion US Dollars by 2050 (O'Neill, 2016).

Antimicrobial Resistance (AMR) occurs when microorganisms causing infections (bacteria, viruses, fungi and parasites) evolve to resist a substance that would normally kill them or stop their growth. As a result, infections are harder to treat, which increases the risk of disease spread and serious illness. A further increase in AMR could lead to significant risks linked to key medical procedures (such as invasive surgeries, chemotherapy, intensive care), threatening modern medicine as we know it (O'Neill, 2016; WHO – Antimicrobial resistance Key facts).

AMR is a natural evolutionary trait of all microorganisms; however, it is accelerated by several factors including: (a) misuse and overuse of antimicrobials in human and veterinary medicine, (b) poor infectious diseases prevention and control measures in healthcare facilities and farms, (c) lack of access to sanitation, hygiene, and medicines and (d) use of heavy metals and biocides in agriculture (WHO – Antimicrobial resistance Key facts; McEwen & Collignon, 2018; Holmes et al, 2016). AMR organisms can be transmitted between people and animals, through the environment or from food origin, having serious health implications for human, animal, and environment (Woolhouse and Ward, 2013; WHO – Antimicrobial resistance Key facts). Therefore, AMR can only be tackled by using a One Health approach to protect all pillars of health and harmonise approaches for more sustainable ways to keep antimicrobials working and improve antimicrobial stewardship.

The scientific community involved in monitoring of AMR is in need for harmonised reliable methods of detection that can be used across all pillars of One Health.

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 Med-Vet-Net Association working together on 47 research projects to address potential and existing risks that originate at the animal-human-environment interface.
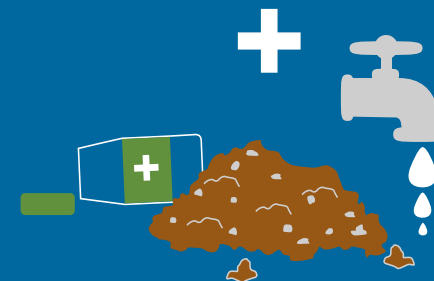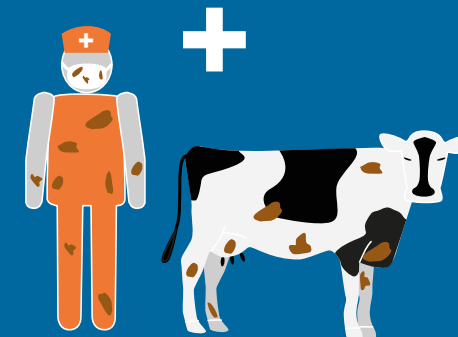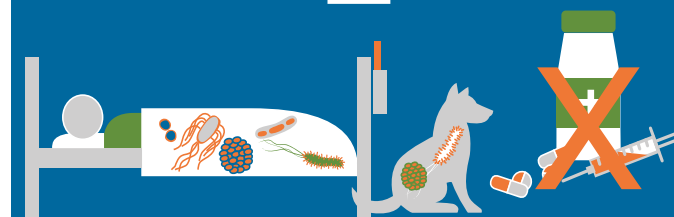
WHAT IS THE IMPART PROJECT?

The IMPART project consists of 13 partners across Europe: The French Agency for Food, Environmental and Occupational Health and Safety (ANSES) in France; Public Health England (PHE) and the Animal Plant Health Protection Agency (APHA) in the UK; University of Utrecht (NCOH), the National Institute for Public Health and the Environment (RIVM) and Wageningen Bioveterinary Research (WUR) in The Netherlands; German Federal Institute for Risk Assessment (BfR) in Germany; Państwowy Instytut Weterynaryjny – Państwowy Instytut Badawczy (PIWET) in Poland; the Statens Serum Institut (SSI) and DTU National Food Institute (DTU FOOD) in Denmark; the Norwegian Veterinary Institute (NVI) in Norway and National Veterinary Institute (SVA) in Sweden; Istituto Zooprofilattico Sperimentale del Lazio e della Toscana (ISS) in Italy.

With these partnerships between human, animal and plant health, food safety and environment protection institutes, the IMPART project aimed to create a unique network to harmonise phenotypic methods for detection of AMR across all pillars of One Health.

At the start of the project, several important knowledge gaps were identified:
- The need for harmonised and reliable methods to detect AMR for monitoring and diagnostic laboratories.
- The lack of harmonised methods for the isolation and detection of Enterobacterales (bacteria found in intestinal tracts of human and animals) that are resistant to colistin and carbapenems.
- The lack of available criteria (epidemiological cut-off values (ECOFFs)) to determine the susceptibility of animal pathogens to veterinary antimicrobials.
- The lack of a cost-effective and rapid method for the antimicrobial susceptibility testing (AST) of *Clostridium difficile*, a bacterium that can cause colitis in human and can be found in various animal species and in the environment.

The IMPART project aimed to address these knowledge gaps by:
- Harmonising the methods of detection for specific types of AMR associated with bacteria relevant to public health, such as colistin-resistant and carbapenemase-producing Enterobacterales.
- Establishing new laboratory test criteria (ECOFF) to improve international harmonisation of the monitoring of antimicrobial resistance in bacterial pathogens from animals and humans.
- Providing a cost-effective, robust and simple method to routinely test the antimicrobial susceptibility of *C. difficile* strains from human, animal or environmental origin.

Adopting a One Health approach is crucial to address AMR and provide sustainable solutions to keep antimicrobials working. Understanding and monitoring AMR across all pillars of One Health will help to improve antimicrobial stewardship which is defined as the promotion and monitoring of judicious antimicrobials use, to preserve their future effectiveness.

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Leader: detection of colistin resistant Enterobacterales
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Leader: detection of carbapenemase-producing Enterobacterales
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Norwegian Veterinary Institute

Project Coordinator and Leader: setting of new epidemiological cut-off values (ECOFFs)
Kees Veldman
Wageningen Bioveterinary Research

Leader: a disk diffusion method for susceptibility testing of *Clostridium difficile*
Sven Maurischat
German Federal Institute for Risk Assessment

IMPART PROJECT OUTCOMES

A key aim of the IMPART project was to harmonise the isolation, detection and characterisation of bacteria and antimicrobial resistance profiles, relevant to human and veterinary public health.

To achieve this goal, the IMPART researchers built a protocol, from a review of the best available genotypic and phenotypic methods to isolate acquired colistin-resistant Enterobacterales from food producing animals and food products. The detection protocol is a two-step screening method involving a PCR amplification and culturing on chromogenic agars media for selective detection and isolation of colistin-resistant Enterobacterales (*mcr*-positive isolates). A performance evaluation study of this protocol was undertaken in a multicentre trial with 11 partners, on meat and caecal samples. The study showed encouraging results, indicating that the pre-screening PCR allows to save time and consumables, is 90% accurate, and that one of the commercial agar plates available on the market has a good sensitivity to detect *mcr*-positive *E. coli* and *Salmonella spp.* in food-producing animals and meat. However, further trials including a broader range of colistin-resistant strains and a larger panel of matrices need to be performed to assess the robustness of the method. More details on this study can be found in the following link: D-JRP1-1.6: Final ring trial summary report (https://doi.org/10.5281/zenodo.4454904)

A multicentre study (11 participants) was also conducted to test the protocol used in most national reference laboratories for the monitoring of carbapenem-resistant Enterobacterales (CPE) in food producing animals and meat. After non-selective enrichment (as in the Decision 2013/652/EU and 2020/1729/EU), the samples were plated on commercially available selective agar media and isolated colonies were used for species identification and AMR testing by phenotypic (microdilution and disk diffusion) or genotypic (PCR) methods. The results from this study showed that the tested selective agar plates performed well on CPE strains but have difficulties to detect low-level carbapenemase producers. There are also no commercial selective agar plates, which are chromogenic for detecting carbapenem-resistant *Salmonella spp*. More details on this study can be found in the following link: D-JRP1-2.6: Final ring trial summary report (https://doi.org/10.5281/zenodo.4452580)

These outputs could be of interest for the European Food Safety Authority (EFSA) and the EU Reference Laboratory for antimicrobial resistance (EURL-AR), to evaluate these protocols in antimicrobial surveillance programmes. Commercial manufacturers could also be interested to improve the performance of their selective media.

Filling in knowledge gaps for antimicrobial sensitivity testing for animal pathogens was also a key outcome of the IMPART project. Although data are available for human pathogens, it is very limited for animal pathogens, which makes it difficult to diagnose and treat infections in animals and ensure antimicrobial stewardship. Therefore, adding to, and improving data for animal pathogens and their antimicrobial resistance profiles has the potential to improve diagnostics, antibiotic prescribing and overall animal health. In close cooperation with The European Committee on Antimicrobial Susceptibility Testing (EUCAST), new epidemiological cut-off values (ECOFFS) were

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established for *Staphylococcus pseudintermedius*, *Staphylococcus hyicus*, *Mannheimia haemolytica* and *Pasteurella multocida* for this project, and are now available on the EUCAST website (www.eucast.org). As a direct result of this work, VetCAST (the veterinary subcommittee of EUCAST) is developing new clinical breakpoints for veterinary antimicrobials.

Finally, providing a cost-effective, robust and simple method to routinely test the antimicrobial susceptibility of *Clostridium difficile* strains was an important aim of the IMPART project. A procedure that can be used worldwide by any laboratory with a scientific or commercial background in *C. difficile* was optimised and validated in this project. For more details, please read the overview:
4. D-JRP1-4.2.: Publication (https://doi.org/10.5281/zenodo.4564519).
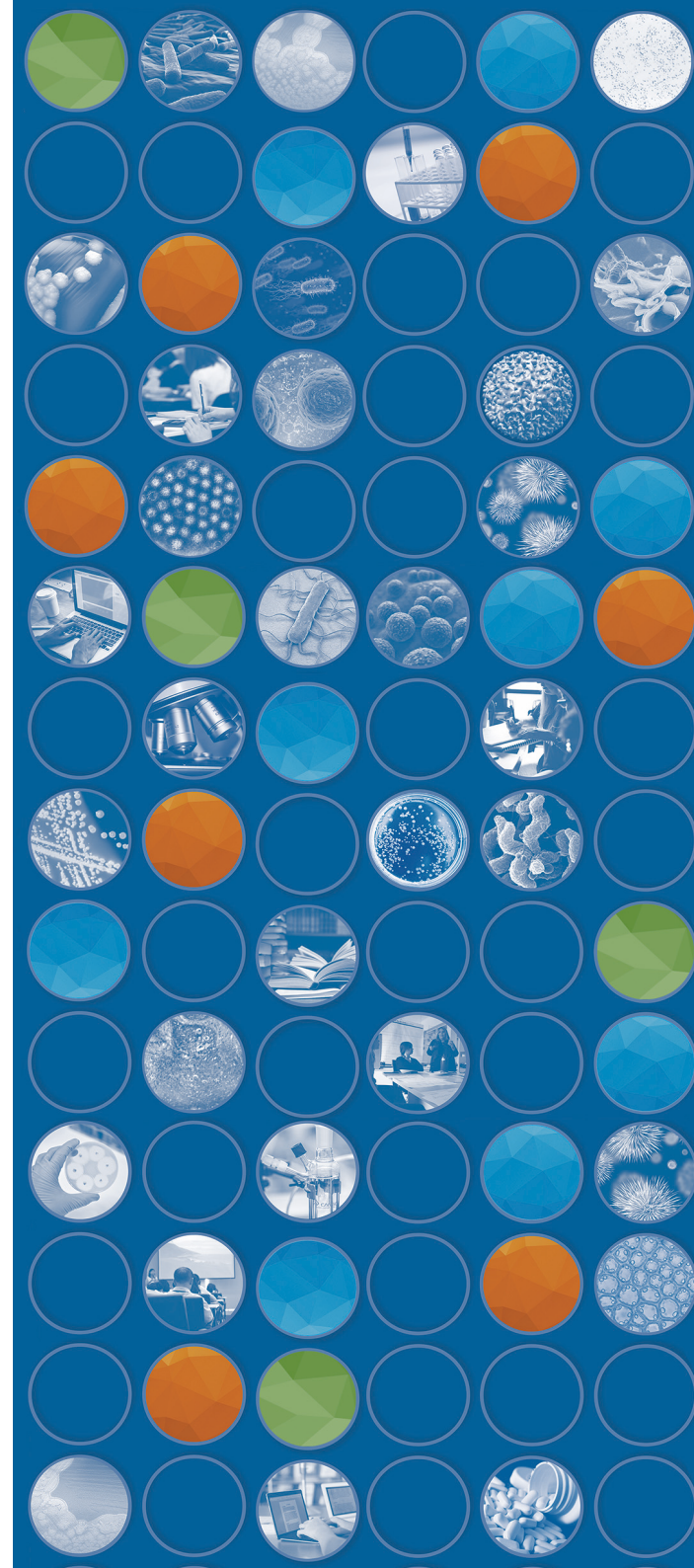
SCIENTIFIC PUBLICATIONS

The IMPART project aimed to develop and harmonise phenotypic methods for detection of antimicrobial resistance, in line with the Commission's Action Plan Against the rising threats from Antimicrobial Resistance: Road Map (updated in November 2016). The research conducted in this project led to many scientific publications on this topic.

Read more in the list below:

Isolation Procedure for CP *E. coli* from Caeca Samples under Review towards an Increased Sensitivity.
Pauly, N., Klaar, Y., Skladnikiewicz-Ziemer, T., Juraschek, K., Grobbel, M., Hammerl, JA., Hemmers, L., Käsbohrer, A., Schwarz, S., Meemken, D., Tenhagen, B-A., Irrgang, A. (2021). *Microorganisms.* 9, 1105.
DOI: https://doi.org/10.3390/microorganisms9051105

Identification of a *bla*VIM-1-Carrying IncA/C2 Multiresistance Plasmid in an *Escherichia coli* Isolate Recovered from the German Food Chain.
Pauly, N., Hammerl, JA., Grobbel, M., Käsbohrer, A., Tenhagen, BA., Malorny, B., Schwarz, S., Meemken, D., Irrgang, A. (2020). *Microorganisms*. 9, 29.
DOI: https://doi.org/10.3390/%20microorganisms9010029

Co-occurrence of the *bla*VIM-1 and *bla*SHV-12 genes on an IncHI2 plasmid of an *Escherichia coli* isolate recovered from German livestock.
Pauly, N., Hammerl, JA., Schwarz, S., Grobbel, M., Meemken, D., Malorny, B., Tenhagen, BA., Käsbohrer, A., Irrgang, A. (2020). *Journal of Antimicrobial Chemotherapy*. 76(2), 531–533.
DOI: https://doi.org/10.1093/jac/dkaa436

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

First Detection of GES-5-Producing *Escherichia coli* from Livestock—An Increasing Diversity of Carbapenemases Recognized from German Pig Production.
Irrgang, A., Tausch SH., Pauly, N., Grobbel, M., Kaesbohrer, A,. Hammerl, JA. (2020). *Microorganisms*. 8 (10), 159.
DOI: https://doi.org/10.3390/microorganisms8101593

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ChromID® CARBA Agar Fails to Detect Carbapenem-Resistant Enterobacterales With Slightly Reduced Susceptibility to Carbapenems.
Pauly, N., Hammerl, JA., Grobbel, M., Tenhagen, BA., Käsbohrer, A., Bisenius, S., Fuchs, J., Horlacher, S., Lingstädt, H., Mauermann, U., Mitro, S., Müller, M., Rohrmann, S., Schiffmann, AP., Stührenberg, B., Zimmermann, P., Schwarz, S., Meemken, D., Irrgang, A. (2020). *Frontiers in Microbiology*. 11, 1678.
DOI: https://doi.org/10.3389/fmicb.2020.01678

Spill-Over from Public Health? First Detection of an OXA-48-Producing *Escherichia coli* in a German Pig Farm.
Irrgang, A., Pauly, N., Tenhagen, BA., Grobbel, M., Kaesbohrer, A., Hammerl, JA. (2020). *Microorganisms*. 8(6), 855.
DOI: https://doi.org/10.3390/microorganisms8060855

A European multicenter evaluation study to investigate the performance on commercially available selective agar plates for the detection of carbapenemase producing Enterobacteriaceae.
Dierikx, C., Börjesson, S., Perrin-Guyomard, A., Haenni, M., Norström, M., Divon, HH., Ilag, HK., Granier, SA., Hammerum, A., Kjeldgaard, JS., Pauly, N., Randall, L., Anjum, MF., Smialowska, A., Franco, A., Veldman, K., Slettemeås, JS. (2022). *Journal of Microbiological Methods.* 193:106418.
DOI: https://doi.org/10.1016/j.mimet.2022.106418

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MEET THE TEAM

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

The IMPART project worked collaboratively to achieve its aims and to produce impactful scientific outcomes which could benefit scientists and policy makers across the globe.

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

- **Animal Plant Health Protection Agency (APHA), United Kingdom.**
  Muna Anjum, Luke Randall, Emma Stubberfield, Olivia Turner, Manal Abu Oun.
  www.gov.uk/government/organisations/animal-and-plant-health-agency

- **French Agency for Food, Environmental and Occupational Health and Safety (ANSES), France.**
  Agnès Perrin-Guyomard, Sophie A. Granier, Marisa Haenni. www.anses.fr

- **German Federal Institute for Risk Assessment (BfR), Germany.**
  Alexandra Irrgang, Mirjam Grobbel, Natalie Pauly, Sven Maurischat.
  www.bfr.bund.de

- **Istituto Zooprofilattico Sperimentale del Lazio e della Toscana (ISS), Italy**
  Alessia Franco, Antonio Battisti. www.izslt.it

- **National Institute for Public Health and the Environment (RIVM), The Netherlands.**
  Cindy Dierikx, Paul Hengeveld, Thijs Bosch. www.rivm.nl

- **National Veterinary Institute (SVA), Sweden.**
  Annica Landén, Boel Harbom, Märit Pringle, Stefan Börjesson. www.sva.se/en

- **National Veterinary Research Institute (PIWET), Poland.**
  Aleksandra Smialowska, Darius Wasyl, Arkadiusz Dors. www.piwet.pulawy.pl

- **Norwegian Veterinary Institute (NVI), Norway.**
  Hanna Karin Ilag, Hege Divon, Jannice Schau Slettemeås, Madelaine Norström, Marianne Sunde. www.vetinst.no

- **Public Health England (PHE), United Kingdom.**
  Matthew Ellington, Neil Woodford. www.gov.uk/government/organisations/public-health-england

- **Statens Serum Institut (SSI), Denmark.**
  Annette Hammerum. www.en.ssi.dk

- **Technical University of Denmark (DTU), Denmark.**
  Jette Seier Kjeldgaard. www.dtu.dk

- **Utrecht University, The Netherlands.**
  Els Broens. (Non OHEJP member.) www.uu.nl

- **Wageningen Bioveterinary Research (WUR), The Netherlands.**
  Yvon Geurts, Teresita Bello Gonzales, Kees Veldman. www.wur.nl